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Dept. of Livestock Services, Harihar Bhawan.*

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Editorial

Livestock farming in Nepal is affected by several important factors. Some of these are : inadequate feed supply, poor genotype, high incidence of diseases, careless breeding and improper husbandry practice. The nutritional aspect of the feed for livestock is always neglected, similarly for obtaining better genotype crossing superior breeds never given due consideration. Improved livestock farming is confined only to the Government farms while in most of the farm-gate level it is yet to be improved. Feeding concentrate in proportion to the milk yield or work performed is never practiced, however, farmers have been found quite aware about animal illness for which the services are also widely available.

Amongst meat animals, best males are mostly selected for slaughter and the neglected or the rejected remaining males are used for breeding purposes. It is a well known fact that the quality of the progeny depends upon the parents from where it is bred. The progeny produced using rejected males will neither grow fast nor become prolific to yield more. The poor genotype in poor environmental and feeding condition is bound to be deteriorated. Indeed it is fact that the improved genotype needs better environment to express its prolificacy and high yielding capability. The genotype and environmental interaction is never considered important in rural livestock farming. The technical aspects of livestock breeding and farming need to be mass communication to every livestock farmers through the media of education and training. Awareness generation, technology transfer and farmers active participation towards improved livestock farming should always be aimed at along with their mass motivation and mobilization. This is the simple livestock extension technique in a common sense for which every technician should be dedicated for. Now time has come to evaluate our past performances and use the knowledge as feed back for future improvement.

Editor-In-Chief

**PREVALENCE OF SALMONELLA INFECTION AMONG CHICKENS IN
PAKHTRIBAS AGRICULTURAL CENTRE,
ITS EXTENSION AREAS AND TWO HMG/N FARMS.**

S. N. Mahato*, K. C. Thakuri**, P. B. Thapa*** and J. N. Yadav***

Summary

A total of 406 serum samples from breeding poultry birds of Pakhribas Agricultural Centre (PAC), Dhankuta, its extension areas, Tarahara Livestock Research Farm (TLRF), Sunsari and Lampatan Animal Husbandry Research Centre (LAHRC), Kaski were examined for the presence of *Salmonella* antibodies. The plate agglutination test using stained *Salmonella pullorum* antigen detected per centages of reactors ranging from 52 at PAC to 67 at TLRF. The organisms isolated from the reactors of the PAC flock were identified biochemically and serologically as *S. pullorum*. Various aspects of *S. pullorum* infection including control measures are also discussed.

Introduction

Poultry keeping plays an important role in the economy of Nepalese hill farmers in general and small farmers in particular. In addition to their usual farm work, they often raise chickens for family consumption and to augment their income.

Productivity of the indigenous chickens is poor. Therefore government and other agencies have made efforts to increase poultry production in villages by introducing

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dual purpose exotic breeds. Several hatcheries have been established to produce exotic chickens for distribution to farmers. The distributed chickens are primarily used as breeding stocks in the villages.

Ideally, the chickens intended for breeding must be produced from flocks which are free from egg-borne diseases. The present study was carried out to investigate the current status of *Salmonella pullorum* infection, an economically important egg borne disease, prevalent among chickens maintained at Pakhribash Agricultural Centre (PAC), its extension areas and two HMG/N farms.

Materials and Methods

Breeding poultry birds from Pakhribash Agricultural Centre (PAC), Dhankuta, Tarahara Livestock Research Farm (TLRF), Sunsari and Lampatan Animal Husbandry Research Centre (LAHRC), Pokhara, Kaski as well as chickens distributed to farmers by PAC were randomly selected for sampling. Blood sample of approximately 2 ml were collected from the jugular vein of 406 chickens from these centres and farm and transported in cold containers to the PAC laboratory. Sera obtained from the blood samples were subjected to the rapid plate agglutination test for the detection of antibodies against *Salmonella* using crystal violet stained *Salmonella pullorum* antigen (Wellcome Reagent Limited, England). *Salmonella* Agglutinating Serum, polyvalent O (A - G) and polyvalent H, phase 1 and 2 (Wellcome Diagnostics, England) were used as positive and negative controls respectively.

Serologically positive four breeding hens from PAC were sacrificed and liver, ovaries, degenerated follicles and caecal tonsil scrapings were collected aseptically for bacteriological examinations. Selenite broth, Tetrathionate broth, Blood agar, MacConkey's agar, Brilliant green agar and Desoxycholate citrate agar were used for the isolation of organisms from the post mortem materials (Anon, 1981). *Salmonella* colonies were identified biochemically using the Mast ID 15 test system and Mast ID Database Codebook, IDM 101 (Mast Laboratories Limited, U. K.). For final identification of the *Salmonella* isolates, antigenic analysis was done by the slide agglutination test using Wellcome Diagnostics *Salmonella* Agglutinating Serum (Carter, 1979).

Results

A summary of the result of the plate agglutination tests carried out on the sera of chickens from different centres and farm is presented in Table 1.

Table - 1. Sero-prevalence of *Salmonella* infection in chickens at different centres and farm.

Centre/Farm	Breed of Chicken	Chickens sample (number)	Positive Samples (number)	Prevalence Rate (%)		
				Breed-wise	Farm wise	Over All
PAC, Dhankuta	New Hampshire	97	66	68		
	Black Australorp	184	81	44	52	
	Light Sussex	12	4	33		
Village flocks in Dhankuta District (chickens distributed from PAC)	New Hampshire	28	16	57	57	
TLRF, Sunsari	Black Australorp	20	13	65		55
	New Hampshire	10	7	70	67	
LAHRC, Pokhara Kaski	New Hampshire	8	4	50		
	Black Australorp	16	12	75		
	White Leg Horn	19	16	74	65	
	Local (Sakini)	12	6	50		

The per centage of the samples positive for *Salmonella* antibodies ranged from 52 at PAC to 67 at TLRF. The overall prevalence was estimated to be 55 per cent.

As can be seen from Figure 1, the per centage of reactors varied from breed to breed. However, the association of breeds with their resistance or susceptibility to pullorum infection cannot be established from this study, particularly among the flocks maintained under different conditions.

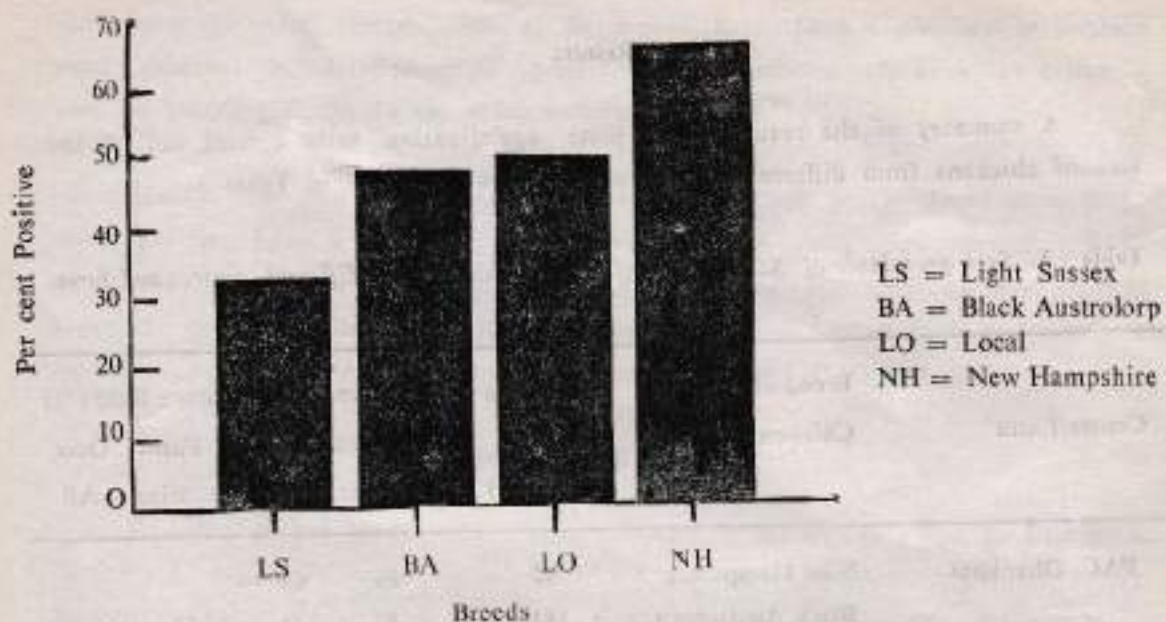


Figure 1: Per centage of reactors of different breeds of chickens.

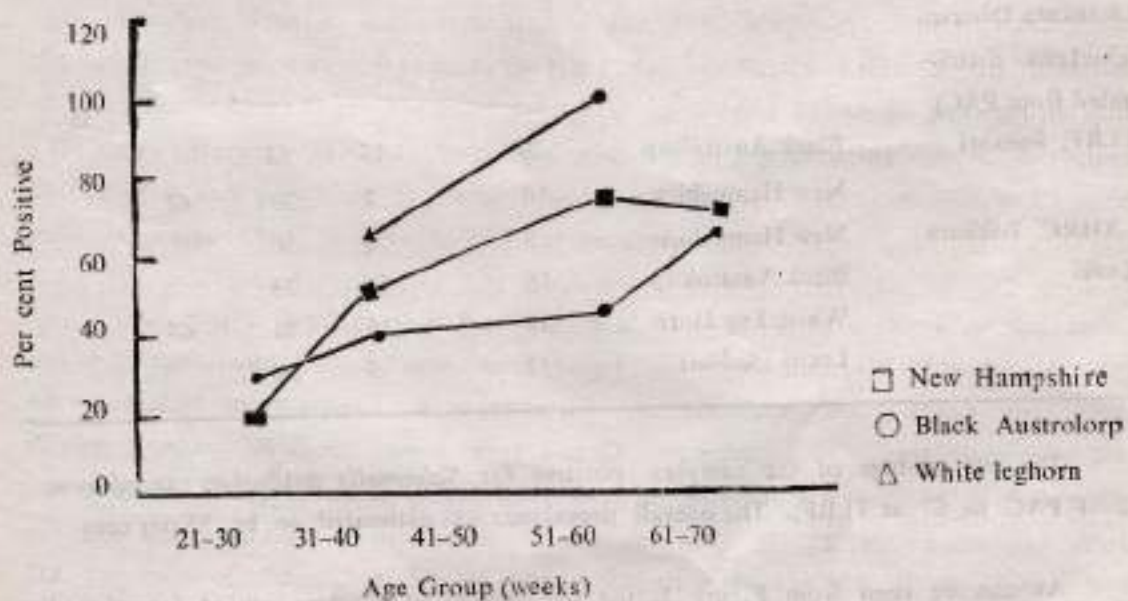


Figure 2: Relation between percentage of *Salmonella* positive chickens and age of different breeds of birds.

From above it is evident that the percentage of reactors increases with age (fig. 2). This suggests that the spread of the disease continues in a flock if carrier laying hens are present.

The cultural and biochemical characteristics and antigenic make-up of the five selected isolates studied are described below under separate sub-headings:

Cultural Characteristics:

On Mac-Conkey's agar, all the isolates produced small, colourless, smooth, round colonies. On Deoxycholate citrate agar, colonies were small, opaque and dome shaped with a central black spot. Brilliant green agar produced small translucent, pale-pink colonies surrounded by a pink zone in the agar. All these characteristics suggested that the colonies were belonging to *Salmonella* species.

Biochemical Characteristics:

All the isolates fermented rhamnose and xylose, and none of them fermented sucrose, melibiose, sorbitol, inositol, amygdalin and dulcitol. They were positive for ornithin decarboxylates and negative for phenylamine, ONPG, urease, malonate and motility tests. All the isolates failed to produce indole. The figure profile (code) generated from the result was 14100. On matching the code with Mast database (Anon. 1987), the isolates were tentatively identified as *S. pullorum*.

Antigenic make-up:

All the isolates agglutinated with polyvalent 'O' (A-G), 9-'O' and 12-'O' confirming that they belong to group D. However none of the isolates agglutinated with 1-'O' and polyvalent H (phase 1 and 2). On the basis of the presence of somatic (O) antigens 9 and 12 and the absence of somatic antigen 1 and flagellar (H) antigens, (Carter, 1979), the isolates were confirmed to be *S. pullorum*.

Discussion

This study indicates that *Salmonella* infection is widespread among chicken in PAC, its extension areas and two HMG/N farms with high prevalence rates. Isolation of *S. pullorum* organisms from post-mortem material of the reactors from PAC confirm

that the antibodies detected in chickens were due to chronic infection with *Salmonella pullorum*. On other farms, the antibodies detected could be either *S. pullorum* or *S. gallinarum* because these both react identically to *S. pullorum* antigens. Occasionally chickens infected with certain other *Salmonella* species, such as *S. enteritidis* may react to the routine pullorum test, however the test detects only 20-30 per cent of birds infected (O'Brien, 1989).

In recent years, a rare serotype, *Salmonella virchow* has been reported in poultry and ducks in the North Eastern Hilly Region of India (Ghosh, 1987). This serotype causes serious economic loss in poultry (Ghosh, Gupta and Verma 1987) and seems to be transmitted through eggs (Ghosh, 1989). Ghosh *et al* (1987) reported that both lighter (White Leghorn) and heavier (Rhode Island Red) breeds are equally susceptible to *S. virchow*, whereas extensive studies have shown that light breeds, particularly Leghorns, are generally more resistant to *S. pullorum* and *S. gallinarum* infection than heavy breeds (Cullen, Gordon, Horry and Jordan, 1979). In the present study, however, the highest per centage of reactors were detected in White Leghorns from LAHRC (Figure-2). This epidemiological evidence is not in support of *Salmonella pullorum* or *S. gallinarum* but *S. virchow* infection at LAHRC. However, further study is needed for its confirmation.

Pullorum disease is widespread throughout the world and is likely to be found wherever chickens are grown. The disease was seen first in the PAC flock in 1978. The whole flock was destroyed and a new pullorum free-flock was established in 1979. The flock was maintained pullorum free until 1985 by systematic testing of all chickens and addition of furazolidone in the feed at a level of 0.04% for ten days for detecting, eliminating carriers and reactors. Further incidence was reduced by compulsory fumigating the incubators with formaldehyde before and after each batch of incubation. Application of these measures were however discontinued after 1985.

Now again, a high per centage of *S. pullorum* carriers has been detected in the PAC flock and there is a high probability of having *S. pullorum* in TLRF and LAHRC. Pullorum disease is primarily a disease of baby chicks in which it assumes an acute, septicaemic, highly fatal form and most of the losses occur during the first two or three weeks of life. Mortality may vary from as low as 2% or 3% to well over 50% (Cullen, Gordon, Harry and Jordan, 1979). In adult poultry birds

the infection is usually symptomless although it may occasionally be manifested by diarrhoea, depression, lack of appetite and paleness of the comb and wattles. The adult carriers especially the hens play an important role in the epidemiology of the disease and becoming a regular source of further spread of infection.

S. pullorum is usually transmitted through the infected eggs laid by carrier hens. The infection is further spread at hatching either by direct contact of infected and healthy chicks, or by the inhalation of infected down or fluff in the incubators (Cullen, Gordon, Harry and Jordan, 1979). Other important sources of infection are contaminated incubators, brooder equipment, nest boxes, litter, feed etc.

Pullorum disease can be successfully controlled by rigid adherence to the detection of carriers by means of the whole blood/serum plate agglutination test and their removal from the flock. The frequency with which the flock should be tested must be determined by the degree of infection encountered. The other complementary measures to break the perpetuating cycle of infection in the flock are initiation of furazolidone therapy if reactors are seen and maintenance of high standard of incubator and hatchery hygiene. Relaxation in any of these measures result in carrier build-up.

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THREE NEW SPECIES OF POULTRY (*GALLUS GALLUS DOMESTICUS*)
CESTODES FROM NEPAL.

Ranjana Gupta* and Jaya Rai**

Abstract

Three new species of tapeworms (Cestodes, Helminth Parasites) of *Gallus gallus domesticus* (domestic fowl) not previously recorded from Nepal are reported. The Cestode parasites are *Amoebotaenia cuneata*, *Cotugnia digonopora* and *Raillietina echinobothrida*. The genus *Amoebotaenia* and its family Dilepididae are reported for the first time in Nepal.

Introduction

Parasitic diseases play a very important role in domestic fowl (*Gallus gallus domesticus*). They in general are chronic, often leading to severe consequences with direct or indirect loss to the national economy. No suitable treatment or control measures can be suggested or put into operation unless the epidemiology of each causative organism is known. It is also essential to know its distinctive characteristic and the condition under which it survives and propagates.

In Nepal, negligible work has been done on the epidemiology of the parasites of domestic fowl (*Gallus gallus domesticus*). The earliest report on poultry cestodes was published by Baczynska in 1941. He described the infestation of *Raillietina cohnii* in *Gallus gallus domesticus*. This is also the first helminth parasite reported from Nepal. In 1954, S. Yamaguti reported that Lopez Neyra in 1944 described *Raillietina*

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cohnii as a variety of *R. tetragona*. In 1943, K.N. Sharma reported *Rallietina parbata* while in 1970 S. S. Singh described *Fernandezia Kantipuri* and in 1988 R. Gupta described *Hymenolepis cariosa*.

Materials and Methods

The cestodes were collected from the intestine of domestic fowl (*Gallus gallus domesticus*) at Kathmandu. The parasites were pressed, fixed in AFA solution (alcohol-formaline-acetic acid solution) and stained in Gower's solution. The prepared slides were studied in microscope and these specimens are deposited in the museum of the Central Department of Zoology, Tribhuvan University Kirtipur, Kathmandu.

Description of the species

1. *AMOEBOTAENTIA CUNEATA* (Linstow 1872).

(Dilepidinae; Dilepididae; Cyclophyllidae; Cestoda.)

Host: *Gallus gallus domesticus*

Description - It is a small worm with an elongate, triangular or roughly fusiform in shape. Collected specimens measure from 1.94 to 3.5 mm long and 1 to 1.5 mm wide. Scolex measures 0.27-0.3 x 0.2-0.25 mm. Scolex bears unarmed suckers measuring 0.08 to 0.09 mm in diameter. Rostellum bottle shaped armed with a single row of 12 to 14 hooks 20 to 32 μ long, proglottides about 20 in number. The proglottides increase in length and width posteriorly. The immature proglottides of collected specimens measure 0.014-0.029 x 0.40-0.51 mm in size. The mature proglottides are 0.09-0.16 x 0.72-1.08 mm in size. There are 12 to 15 testes in transverse row placed behind the ovary close to the posterior border of the segment. The testes measure 0.02-0.027 x 0.03-0.04 mm. The cirrus pouch is comparatively small in the extravascular field. The genital pore alternate regularly and is located at the extreme anterior point of the segment margin. In the mature proglottides the distance of genital pore from the anterior border of the segment is 0.013-0.02 mm. Ovary is single and measures 0.04-0.05 x 0.51-0.6 mm in size. Vitelline gland is compact, median and measures 0.04-0.09 x 0.1-0.13 mm. Vagina lies posterior to the cirrus pouch. The gravid proglottides measure 0.163-0.23 x 1.22-1.42 mm in size. Uterus is saccular and

the eggs are without egg capsules. The eggs measure 0.01 - 0.014 mm. in diameter. In the gravid proglottides the distance of the genital pore from the anterior border of the segment is 0.027 - 0.04 mm. (Fig. 1).

2. *COTUGNIA DIGNOPORA* Pasquale, 1890

(Davaineinae; Davaineidae; Cyclophyllidae; Cestoda)

Host - *Gallus gallus domesticus*

Location - Small intestine.

Locality - Kathmandu

Description - The worm measures upto 107 mm long.

The scolex of collected specimens measure 1.22 - 1.45 x 1.18 - 1.42 mm. in size. The suckers are unarmed and 0.39 to 0.43 mm. in diameter. The rostellum bears two rows of small hooks. It measures 0.34 - 0.35 mm. long and 0.23 - 0.24 mm wide. The neck is 1.25 - 1.32 mm. long (Fig. 2).

The proglottides are very much wider than long. The mature proglottides measure 0.23 - 0.89 mm. long and 1.65 - 2.31 mm broad. There is presence of double set of genitalia. (Fig. 2). Testes are present in intervacular field. The testes follicles measure 0.041 - 0.068 x 0.02 - 0.04 mm in size. Genital pores bilateral and they are situated at the distance of 0.136 - 0.38 mm. from the anterior end of the segment in the mature proglottid. Ovary is just medial to excretory stems. They measure 0.099 - 0.17 x 0.07 - 0.1 mm in size. The vitellaria measures 0.07 - 0.1 x 0.05 - 0.08 mm. The gravid segments measure 2.77 - 3.3 x 3.5 - 4.2 mm. in size and the distance of genital pore from the anterior end of the segment is 1.55 - 1.68 mm. The gravid uterus is replaced by parenchymatous egg capsules consisting one egg per capsule (Fig. 2). The eggs measure 0.033 - 0.04 mm.

3. *RAILLIETINA ECHINOBOTHRIDA* (Megnin, 1881)

(Davaineinae; Davaineidae; Cyclophyllidae; Cestoda)

Host - *Gallus gallus domesticus*

Location - Small intestine

Locality - Kathmandu.

Description - The body of mature specimens measure upto 26 cm. in length.

The scolex measures $0.27-0.32 \times 0.15-0.25$ mm in size. The suckers are round in shape; armed with 8-10 rows of hooks, and measure $0.06-0.09$ mm in diameter. Rostellum are armed with 2 rows of 200 to 240 hooks (Fig. 3).

The immature proglottides measure $0.078-0.19$ mm in length and $0.13-0.97$ mm in breadth. The mature proglottides are more wider measuring $0.16-0.59 \times 0.96-1.38$ mm in size (Fig 3). Testes are 20 to 30 and occasionally counted even as many as 45 in number. Genital pore is unilateral and located at the middle or posterior to the middle of the segment margin. In mature proglottides the distance of genital pore from anterior border of the segment is $0.075-0.28$ mm. The testes measure $0.027-0.048 \times 0.02-0.03$ mm. Ovary is single and pretesticular. Vitelline gland is compact, median and postovarian. Ovary measures $0.136-0.177 \times 0.05-0.08$ mm in size. The vitellaria is $0.045-0.06 \times 0.03-0.04$ mm. Vagina is situated posterior to the cirrus pouch. There are egg pouches in the gravid segments formed by the breaking of uterus (Fig 3). Each egg capsule (egg pouch) contains 6 to 12 eggs. The eggs measure $0.13-0.14$ mm. Segments measure 2.36 to 3.15 mm in length and $0.94-1.87$ mm in breadth. Posterior gravid segments frequently become constricted longitudinally through median line to form windows in the centre. In gravid proglottides the distance of genital pore from the anterior border is $1-1.4$ mm.

Discussion

All the domestic fowls dissected for parasites collection were obtained from Kathmandu valley. Until now four cestode species have been reported in *Gallus gallus domesticus* in Nepal viz. *Railletina cohnii* (H. Baczynska 1914) to which Lopez-Neyra in 1944 described as a variety of *R. tetragona*: *Railletina parvata* (K.N. Sharama 1943), *Fernandezia Kantipuri* (S. S. Singh 1970) and *Hymenolepis carioca* (R. Gupta 1988). Three additional species have been reported as described in the present paper. Keys from Yamaguti (1959, (1976) have been followed to identify the parasites. The description of the present specimens are similar with most of the characters of the genus and species given by Everett E. Wehr (1967).

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**INITIATION OF TISSUE CULTURE AND ITS USE IN DISEASE
DIAGNOSIS AND VACCINE PRODUCTION IN
VETERINARY MEDICINE IN NEPAL.**

S. P. Gautam*

Introduction

Tissue culture is concerned with the study of cells / tissues and organs maintained or grown in - vitro for more than 24 hours. Tissue culture generally denotes growing of cells or maintenance of tissue fragments in - vitro where as cells are no longer organized into tissue (Fedoroff. S., 1967).

It is an old technology for developed countries. It has become a daily routine work in the field of quick disease diagnosis, mass vaccine production (Turner, G. S. *et al.* Plowright, W. *et al.* 1969), conducting viral research works, drugs / chemicals / toxins trials and many other field (John, P. 1979). This technology is completely new in the field of veterinary medical science in Nepal as there is no evidence of publication or research conducted in animal tissue culture. However there is some evidence of plant tissue culture work undertaken in Godavary.

After Completing a short term training by the author in 1984, an environment and facilities suitable to produce tissue culture in Biological Products Division, Tripureswor was created. In the laboratory chicken embryo fibroblast, primary cultures of rabbit kidney, lamb kidney and continuous cell lines of vero cells were successfully produced. Some of the tissue cultures were used in the field of disease diagnosis mainly in two poultry diseases such as Ranikhet and Fowl Pox. Towards vaccine production the combined method of production and control protocol of Rinderpest cell culture vaccine adopted

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by Animal Virus Research Institute, Pirbright, England (1983) and Indian Veterinary Research Institute, Mukteswar, India (1984) were followed and produced one batch of vaccine using lamb kidney primary cell culture which is in trial phase.

In all of the processes Analytical Reagent Grade Chemicals from BDH Chemical, Difco and Sigma Laboratories were used. The medium used in the production was Medium-199 and Foetal Calf Serum exclusively from Flow Laboratory.

Materials and Methods

Disease Diagnosis

Material and Methods using chicken embryo fibroblast tissue cultures for disease diagnosis are described below.

- Eleven days old healthy embryonated eggs of Chicken were procured from Livestock Development Farm, Khumaltar.
- Eggs cleaned, swabbed with Tr. of Iodine, candled and air space marked.
- Eggs broken and opened at air space and embryos collected aseptically in chilled hanks balanced salt solution (H. B. S. S.) using sterilized instruments.
- The Viscera, appendages and head were removed and rest of the embryo collected in a beaker containing H. B. S. S. and washed repeatedly.
- The organs were cut into small pieces using scissors and all the procedure of washing, trypsinization, centrifugal washing, packed cell volume (PCV) collection for primary culture were followed as mentioned by different authors (John Paul, 1979), Akio S. WHOTRS, 1970) and protocols.
- The PCV collected were dissolved in Medium-199 containing 10 per cent Foetal Calf Serum at the rate of 1 ml PCV/250 ml of medium.
- Two ml of seeded medium were distributed into test-tubes and incubated at 37°C.
- The incubated cultures were observed daily for attachment, growth contamination, change in pH, etc.
- After 36 hours of incubation test-tubes showing good growth of chicken embryo fibroblast monolayer without any evidence of contamination were selected and grouped in to two groups A and B.

For each group A & B ten test-tubes were kept to study the effect of Virus Ranikhet R₂ B strain & Fowl Pox strain respectively.

In Group A —

- Out of ten test-tubes cultures, seven test-tubes were inoculated with 0.1 ml of 1:100 dilution of R₂ B strain of Ranikhet disease virus after pouring - out the media and kept in the incubator for 30 minutes for virus adsorption. Three test-tubes were kept as control.
- After adsorption of virus, media containing 5 per cent serum was changed in all ten tubes and incubated at 37°C.
- Test-tubes observed daily for any evidence of cytopathic effect (CPE) of the virus on chicken embryo fibroblast cell culture and compared with the control tubes.
- The progress of CPE from rounding of cells, clustering, sloughing off or detachment etc were observed daily.
- The test-tubes showing clear evidence of CPE were stained with Haematoxyline and Eosine (H & E) stains along with the control tube.
- Stained cultures were fixed and preserved using DPX mount in glass slide.

In Group B —

- Out of ten-tubes cultures seven of them were inoculated with 0.1 ml of 1:100 dilution of Fowl Pox Vaccine virus from Biological products Division Tripureswor.
- Rest of the procedure of adsorption of virus, incubation, observation preservation etc were followed in the same manner as already mentioned above in Ranikhet R₂ B strain.

The micro-photograph of the cell cultures showing CPE of R₂B and Fowl Pox along with control taken at 10 x 100 x of microscope are as shown in the fig 1,2,&3 respectively.



Fig. 1 CPE of R₂B Strain on Chicken Embryo Fibroblast (100 × 10x)

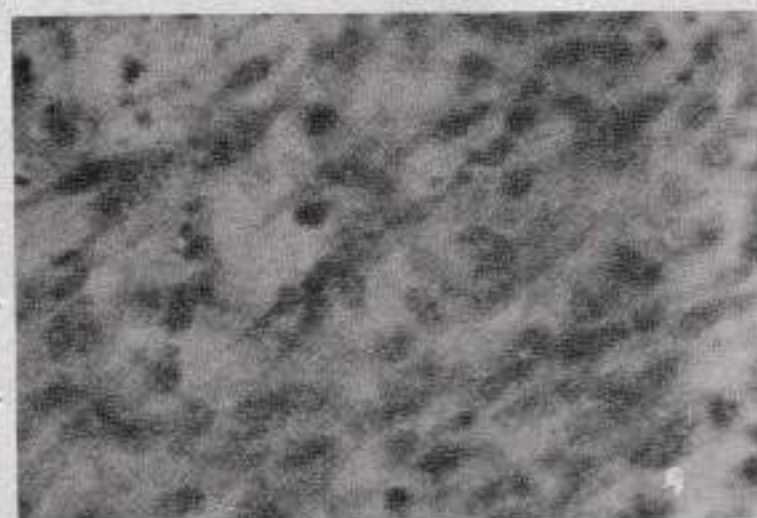


Fig. 2 CPE of Fowl Pox on Chicken Embryo Fibroblast (100 × 10x)



Fig. 3 Normal Chicken Embryo Fibroblast (100 × 10x)

II Vaccine Production:

Cell Culture Rinderpest Virus Vaccine Production:- The production of Rinderpest cell culture vaccine in many countries is done by primary culture of bovine calf kidneys (Row, L. 1987, WHO-TRS, 1970, Thatcher, M. 1988). But in Nepal bovine kidneys can not be harvested for the production of vaccines. Some report of using Vero cell lines (Sonoda A. Japan) and primary culture of lamb kidney (protocol, IVRI - India, Row L.W., Thatcher, M.) are available for the production of Rinder pest virus vaccine. Therefore, it is decided to adopt the primary culture of lamb kidney for this purpose for the first time.

Materials and methods used for the trial production of cell culture Rinderpest virus vaccine is as follows:

- Two weeks old healthy suckling lamb was purchased from the market.
- Lamb was kept in isolation and observation for two days, then stunned, deskinsed and both the kidneys were harvested using sterilised instruments and placed in a beaker containing chilled H.B.S.S.
- The protocol of production and control of Rinderpest cell culture vaccine adopted by Animal Virus Research Institute, Pirbright, England and Indian Veterinary Research Institute, Mukteswar, India were followed. The cultures were grown in Polystyrene Roller bottle, Poly-sterelene flask, Roux flask and test tubes. The vaccine seed used was Kabete 'O' strain of Rinderpest virus obtained from Pirbright, England.
- Daily observation of infected and control cultures were carried out and initiation of rounding the cells, clustering and other effects were noted.
- When the infected cultures were showing 75 per cent CPE or more in eleventh day of infection the fluid was pooled together.
- The equal volume of stabilizer (5 per cent LAH and 10 per cent sucrose) were mixed with the harvested fluid.
- Final mixture was filled into sterilized glass vials at the rate of 0.5 ml/vial.
- These vials were subjected for freeze drying and stored at 20°C of the freezer and some of them sent for further tests and trials.

Discussion

Animal tissue culture technology which is well known for its diversified use in many countries will be beneficial for Nepal if properly adopted. A boost in Nepalese economy by saving lot of foreign currencies may be targetted if this technology could be used in different field of disease diagnosis, viral vaccine production and virus isolation research works, etc, in Nepal.

The above trials and studies indicate that this technology could be successfully used in disease diagnosis and vaccine production and save the foreign currencies invested every year for sending samples, procuring tissue culture viral vaccines Rinderpest, Swine Fever, Mareks Disease etc. Further, it can even generate foreign currencies by selling the surplus vaccine to the neighbouring countries. The life of the animal population saved using the vaccines produced adopting tissue culture will further help in increasing the national economy.

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MALICIOUS POISONING IN RHINOCEROSSES-A CASE REPORT

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Abstract

Two rhinoceros of a local zoo were found lying dead early in the morning on 27 January 1990. The post-mortem examination showed lesions of acute poisoning and the toxicological analysis confirmed the presence of Zinc Phosphide in autopsy materials. This paper reports the autopsy findings and the concentration of Zinc Phosphide in different organs/tissues of these protected wild animals. The intestinal tissue was found to contain the highest concentration of the poison.

Introduction

One horned rhinoceros (*Rhinoceros unicornis*) is the secondlargest terrestrial creature alive today and is kept in the list of endangered species. Despite protective laws, it has been hunted extensively and poaching continues, mainly because of myths that the horn contains an aphrodisiac and other body parts also have magical qualities [Eltringham (1984); Felix (1983); The World Book Encyclopedia (1984)]. Eltringham (1984) reports that the rhino horn has large market in the Far East and in North Yeman. Although the market price of the horn of *R. unicornis* is about US \$ 1800/kg [Chakravarty and Baruah (1985)], it was on sale at unbelievable price of US\$ 17000/ kg [Eltringham (1984)].

Martin (1983) reported that traditionally rhino horn was used in Unani and Ayurvedic medical practices while the skin was used in battle field. Rhino products

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like urine, dung, horn, skin, hoof, meat, bone, blood etc. are used as aphrodisiac, antidotal, analgesic, cardiac stimulant, antiinflammatory, anticonvulsant, purgative, carminative, muscle relaxant, antiemetic, antiallergic and antishock therapy. He further reported that rhino products were also used in development of full-breast in female, stop bleeding, haemorrhoids, paralysis, polio, erysipelas, measles, influenza, leprosy, whooping cough, typhoid, pleurisy, food poisoning, asthma, laryngitis, tonsillitis, caruncles, abscess, infantile, night blindness, high blood pressure, skin diseases and to keep away bad spirit too.

The anatomical study of rhino horn indicates that it is a kind of epidermal growth of skin in the form of modified hairs [Chakravarty and Baruah (1985); Kent (1978)]. The actual medicinal value and its pharmaceutical effect within the system has not yet been reported. But the presumed medical properties of rhino horn are so highly regarded in the East, particularly in China, and there is little chance that immediately traditional doctors will discontinue its use in the name of conservation [Eltringham (1984)]. Consequently, the poachers are adopting various means of killing the protected animals.

Zinc phosphide is a commercially available rodenticide poison. Fatal cases of poisoning from zinc phosphide have been reported in man and in nearly all species of domestic animals and poultry [Clarke, Hervy, Humphreys (1981); Franklin (1988); Singh and Pahuja (1985)]. Various attempts have been made to study the toxicity of zinc phosphide in domestic animals and man. The gaseous phosphine (PH_3) is reported to be the toxic principle which is rapidly released through hydrolysis in dilute solution of hydrochloric acid of stomach but slowly in water. Therefore, zinc phosphide is more hazardous amongst horses (rhino has also similar digestive system) than in ruminants (rumen has alkaline pH) [Blood, Radostits and Henderson (1983)]. The cause of death due to phosphine poisoning in man is asphyxia [Dreisbach (1983)]. Fitzpatrick et al (1955) have given detail account of the postmortem findings in various species but not in rhinoceros.

The lethal dose of zinc phosphide for man is 1mg/Kg body weight [Dreisbach (1983)] while for cattle, sheep, pig, goat, dog and cat it is between 20-40 mg/kg [McGirr (1953)] but Fitzpatrick et al (1985) mentioned that the fatal single dose

orally is approximately 60 mg/kg and there is little species variation in this regard. In case of heavy intoxication, zinc phosphide may kill the animals immediately after ingestion and the symptoms are frequently not observed [Jones (1974)].

Commercial zinc phosphide contains two fractions, one rapidly and the other slowly attacked in the gut [McGirr 1953], the later fraction is of considerable importance because its refractory nature allows the poison to be detected chemically after death. It has been proved that zinc phosphide is resistant to natural decomposing forces and in a homicide case it has been detected in the stomach after 8 days of burial [Tiwari (1965)]. Singh and Pahuja (1985) have estimated the concentration of phosphorus and zinc in different organs/tissues in a fatal case of zinc phosphide poisoning of a couple but the authors could not find any report about the chemical analysis of zinc phosphide in visceral organs of rhinoceroses. The present case report will be able to draw the attention of world's zoos and wildlife reserves about the possibility of such malicious poisoning, especially in endangered species of wild animals.

History of the Case

Two rhinoceroses ageing 15 and 6 years, sex female and male respectively, kept in two separate enclosures at Central Zoo of Nepal, were normal till 5:30 PM on 26th Jan 1990. They were fed on usual routine food. Both the animals were found dead at 6:00 AM on 27th Jan 1990. There were no remnants of bait or poison packets. The horn of male rhinos was cut and taken away by the poachers leaving behind a heavy bleeding.

Material and Methods

Post-mortem examination of the female rhino was carried out from 4:00 PM to 9:00 PM on the same day in a systematic manner where as post-mortem examination of male rhino was carried out from 10:00 AM to 3:00 PM on the next day. Autopsy findings were noted and were photographed where appropriate.

Preliminary test for zinc phosphide was done in viscera and stomach content by treating with sulphuric acid and detecting the release of phosphine gas with mercuric

chloride paper. The phosphate was further confirmed by oxidizing with acidified potassium permanganate and testing with ammonium molybdate and 1,2,4-aminonaphthol sulphonic acid reagent. Rinmann's green test was performed to confirm the presence of zinc. The quantitative estimation of zinc phosphide was done colorimetrically with known standard of potassium dihydrogen phosphate by Fiske and Subbarow method.

1ml of 1N KH_2PO_4 solution $\rightarrow$ 1.818 mg. of $\text{Zn}^{+2}\text{P}^{3-}$

Results and Discussion

Autopsy examination revealed identical changes in both the cases. Externally animal was lying down on lateral recumbency. Tympany was remarkable and froth was evident at the nostril. There was little bleeding from the nose as well as from rectum. The rectum was prolapsed. Visible mucus membranes of eye, rectum, nostril, lips and tongue were heavily congested. The pupils were dilated.

On opening of the stomach there was a characteristic smell of something between garlic and rotten fish. Glandular portion of stomach wall was severely congested and inflamed. Ingesta was containing some blackish pastelike material. At adjacent to stomach wall, the ingesta was covered with whitish material with some blackish spots. Small intestinal mucosa was highly congested, inflamed, and was filled with gas and bluish black fluid. The mesentric blood vessels were highly congested. The liver was highly inflamed, fragile and congested. Both the kidneys were also congested. The spleen was inflamed and spongy with patchial haemorrhage over the surface.

Internal wall of the thoracic cavity showed ecchymotic haemorrhage at several parts. The lungs were also highly congested with ecchymotic haemorrhage and interlobular oedema. All the blood vessels of the heart were highly congested and patchial haemorrhage was observed on endocardium.

Qualitative test for zinc phosphide gave positive result in stomach content, intestine, spleen, liver, kidney, lungs, and heart of both the rhinoceroses.

The values of residual zinc phosphide obtained in various specimen are noted as below:

Materials	Residual Zn_3P_2 (mg/100 gm)	
	Female rhino	Male rhino
Small intestine	3.6	4.545
Liver	0.259	0.3305
Spleen	0.1165	3.232
Kidney	0.101	0.101
Lungs	0.0505	0.04545
Heart	0.1818	0.1212

The blackish pastelike unmixed material found in the ingesta gave strong positive result for zinc phosphide while other portion of the stomach content was hardly positive. Due to this heterogeneous mixing nature of the stomach content it was not considered for quantitative estimation of the poison. The presence of considerable amount of blackish pastelike material in the stomach also suggests that the poison was given in a very high dose.

Since the amount of zinc phosphide given to the animals was very high, death occurred within 12 hours and the findings that intestinal tissue contained highest concentration of zinc phosphide is consistent with the expectation.

Pathological changes caused by zinc phosphide intoxication in the rhinoceroses were not different from those reported in other species. Therefore, it was evident that gaseous phosphine acted similarly and produced similar effects in rhinoceroses as it did in other animals and man. However, it was not clear that the traditional presumption as the horn and other bits and pieces of rhinoceroses can cure a variety of illness and can also be used as an antidote in many poisoning cases; why these rhinoceroses failed to produce antidotal substance in their own? The question remained yet to study.

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**A SURVEY REPORT TO ESTIMATE PET DOG NUMBERS AND
DETERMINE PEOPLES ATTITUDES TOWARDS RABIES
CONTROL PROGRAMME IN KATHMANDU VALLEY** $\angle$

A. R. Mercy* D. D. Joshi** P. Pathak*** T. N. Vaidya**** and
S. L. Shrestha*****

Summary

A survey was conducted using questionnaire in the Town Panchayats of Kathmandu Lalitpur and Bhaktapur and interviewed 100 householders in each municipality. Among the information obtained, it was found that approximately four in ten households owned one or more dogs. By using the dog and human population figures from the survey, it is estimated that the number of pet dogs in the three municipalities could be in the range of 20 – 30 thousand. There was a high awareness of rabies in the community and expressed favourable response towards the proposed rabies control programme. Ninety seven per cent of dog owners indicated their willingness to take their dog in a clinic for vaccination. Only one in ten people said they would not like the campaign to remove stray dogs from their area. Information on the likely impact to be resulted with the advertisement through the various media was obtained and discussed.

Introduction

It was initiated to carry out a mass vaccination programme to control canine rabies in the Kathmandu Valley immunizing the majority of pet dogs, followed by

$\angle$ This study was undertaken during 1983 by the authors.

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a systematic campaign to remove unvaccinated dogs. No factual data was available on the statistics of dogs population in the Kathmandu Valley, although an estimate of 40-50,000 had been suggested. It was also believed by some people that only 50 per cent of this number owned by individual households—the rest being either stray or "community owned dogs".

Since the mass vaccination campaign was to rely on the voluntary submission of pet dogs to vaccination clinics for anti rabies vaccination, it became necessary to find out some information about the dog population and the community attitude towards the proposed programme. Accordingly a survey was carried out in the Town Panchayats of Kathmandu, Lalitpur and Bhaktapur districts.

Methodology

A survey was carried out during August 1-4, 1983 interviewing 100 households in each of the three Town Panchayats. A standard questionnaire was used as survey instrument.

Ten areas in each town Panchayats were chosen at random and three enumerators were sent in different directions to visit every third house for interview and complete the questionnaire. Ten questionnaires were completed for each area. A list of the areas visited is follows:

Kathmandu - Thapathali, Battisputali, Chabhel, Maharajgunj, Bishalagar, Kirtipur, Tahachal, Thamel, Baneswor, and Balaju.

Lalitpur - Jawlakhel, Jhamsikhel, Sanepa, Kupondole, Satdobato, Lagankhel, Mangal Bazar, Sankhamul, Pulchok, and Dhobigat.

Bhaktapur - Kwathong Tole, Thulchhe Tole, Etachhe Tole, Taumadi Tole, Enacho Tole, Kwachhen Tole, Kulachhe and Malacho, Durbar Square, Dudhparai Tole, and Tekhacho Tole.

Results and Discussion

The survey findings indicated that the overall awareness of rabies amongst the people interviewed seemed high with 56 per cent of people knowing that dog bites

are the main source of human rabies cases, despite only 36.5 per cent of respondents saying they had heard of the disease.

Dog ownership was highest in Kathmandu with 57 per cent of interviewed households, in Lalitpur and Bhaktapur it is 39 and 28 per cent respectively. Overall 41.3 per cent of households maintained one or more dogs in the valley. In Lalitpur and Bhaktapur towns, the number of dogs in individual household varied from 0 to 4 with an average of 1.4. Among the respondents 64.6 per cent of the households claimed that their dogs were tied up at night.

Eighty four per cent of people having pet dogs indicated that they would like to vaccinate their dogs against rabies when the cost is Rs. 5/-

Some 97 per cent of the dog owners said that they would take their dog to a free rabies vaccination clinic located in their ward. Almost 48 per cent of the dog owners indicated that 7-9 AM would be the most convenient time to take their dog to a clinic for vaccination. This information would be useful in setting up the operation hours of mobile clinics.

About 28 per cent dog owners said their dog had a collar, although 84 per cent informed that they would like to buy one if it is compulsory to have a collar after vaccination for rabies and the cost is comparatively cheaper, that is Rs 5 only. It is noted that higher the price lower the interest.

With respect to the community's attitude to a campaign to destroy unvaccinated/stray dogs, it would appear that only nine per cent of people would object.

It had been thought that ward and community group meetings would be a useful method of informing the public about the vaccination programme. Surprisingly some 55 per cent of the people indicated that they have seen some advertisement about rabies in the daily newspaper "The Gorkhapatra".

By using these findings the number of people and pet dogs in the surveyed districts, a ratio of dogs: people have been calculated. This ratio is 0.065, 0.069 and 0.042 for Kathmandu, Lalitpur and Bhaktapur Town Panchayats respectively. So the estimated pet dog population in these town panchayats are 19,480, 6,993 and 5,681, respectively summing up to 32,154 in the valley.

It is emphasised that this figure may be an overestimate due to "sampling error" resulted with selecting ten overpopulated areas in each town. If less densely populated areas would have been selected the true dog: people ratio would have been less than the figure obtained. It was not possible to estimate the the number of stray dogs from the information obtained in this survey.

Acknowledgement

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LACTOSE INTOLERANCE : A REVIEW

Nagendra P. Shah*

Lactose intolerance: "Lactose intolerance" is a condition in which lactose, the main sugar of milk, is not metabolized into its monosaccharides glucose and galactose. Since lactose is a disaccharide, people can not absorb it directly without splitting into monosaccharides by the enzyme lactase. The lactase is located in the brush border of the mucosal cells of the small intestine. Lactose intolerant individuals suffer from the deficiency of this enzyme; when the amount of lactose ingested is higher than the metabolic capacity of the available intestinal lactase, some of the lactose remains undigested and passes from small intestine to the colon where two processes follow. The first process is physical where water is drawn from the tissues into the colon by osmotic action. The second process is biochemical: some of the undigested lactose is fermented by microflora in the colon producing organic acid and gas. The fermentation products and the large amount of water drawn into the intestine are largely responsible for the symptoms commonly associated with lactose intolerance such as, bloating, flatulence, cramps, and diarrhoea (Kretchmer, 1972).

Definitions: The definition of three often misused terms relating to the digestion of lactose is as follows:

(a) **Lactase deficiency** is a condition of low intestinal lactase activity. Two forms of lactase deficiency have been reported—primary and secondary. The primary form of lactase deficiency is the condition which results when the brush border of the small intestine has nearly stopped production of lactase. This form of lactase

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deficiency is genetic in nature and approximately 70 per cent of the world population is afflicted with this condition. Secondary lactase deficiency usually results from nutritional deficiency or disease of the digestive tract and is usually temporary.

(b) **Lactose malabsorption** is the body's inability to metabolize some or all of lactose due to lactase deficiency. The lactose which is not absorbed by lactase deficient individuals results in its malabsorption. Some persons who are diagnosed as lactose malabsorbers may develop the discomfort symptoms associated with lactose intolerance.

(c) **Lactose intolerance** is the symptomatic response to the consumption and malabsorption of lactose. Not all lactose malabsorbers show symptoms of lactose intolerance and there are varying degrees of tolerance among lactose malabsorbers. About 30-95 per cent of the lactose malabsorbers experience the discomfort of lactose intolerance after ingestion of about 25 g. of lactose (equivalent to 500 ml of milk).

Milk allergies are associated with milk-protein hypersensitivity and must be distinguished from **lactose malabsorption** and **lactose intolerance**.

Measurement of lactose intolerance: It is possible to measure the appearance of clinical symptoms such as flatulence and diarrhoea after the ingestion of 50 g. of lactose in water. Another method to measure lactose intolerance is to feed 50 g. of lactose and measure the rise in blood glucose level following post ingestion. Lactase deficient persons can not metabolise all the lactose and their blood glucose level does not rise. A lack of rise in blood glucose level of less than 20 mg/dL is indicative of lactose intolerance.

Lactose intolerance can also be measured by breath hydrogen test (BHT) which measures the hydrogen concentration in the breath sample. Malabsorbed lactose passes undigested to the colon where it is fermented by microflora resulting in production of hydrogen and carbon dioxide gases. These gases pass rapidly from the colon to the lungs and appear in the exhaled air. The subjects are classified as lactose intolerant on the basis of a rise in breath hydrogen concentration to >200 ppm, after ingestion of 50 g. of lactose.

Prevalence of lactose intolerance: Among adults, lactase deficiency develops from a lack of continued substrate availability which may result in gradual decline in the enzyme production following withdrawal of milk from the diet as a child grows older. High incidence of lactose malabsorption and intolerance are generally found in the traditional nondairying areas of the world where adults obtain little or no milk. These areas include: Southern and Eastern Asia, Indian subcontinent, and Africa. It is estimated that about 70 percent of the black Americans, 67 per cent of the Indians, 98 per cent of the Thais, 97 per cent of the Chinese, 99 per cent of the Japanese, 72-99 per cent of Africans, and 10-15 per cent of the Caucasian people are afflicted with the problem of lactose intolerance (Alm, 1982). Prevalence of lactose intolerance is low only in those regions of the world where there is a long history of dairying.

Importance of lactose intolerance: It is estimated that about 70 per cent of the world population are unable to properly digest and utilize fresh milk. This has important implication for the dairy industry as well as for human nutrition considering milk as an excellent source of protein, calcium, and other nutrients such as vit B₁₂, riboflavin and phosphorus. Lactase-deficient persons avoid milk and other dairy products high in calcium because of malabsorption of lactose and the accompanying intolerance symptoms and therefore, may consume suboptimum levels of calcium. In lactose intolerant persons, ingestion of milk can cause diarrhoea resulting in decreased absorption of nutrients in general. The problem becomes more critical if lactose intolerant persons develop osteoporosis. Indeed, a relationship between lactase deficiency and osteoporosis has been reported. Birge *et al.* (1967) observed that nine persons, who excluded dairy products from their diet because of gastrointestinal upsets due to lactose intolerance, had developed osteoporosis. Therefore, withdrawal of milk and lactose-containing dairy foods from a large population of lactose intolerant people is a matter of great concern for health professionals, policy planners, as well as for the dairy industries.

Another important area of concern is, the lactose intolerance in population of the developing countries where poverty as well as severe protein energy malnutrition exist. Studies have indicated that malnourished populations have a very high prevalence of lactose intolerance. UNICEF, FAO, WHO, WFP, and other agencies sponsor many nutritional programs including an introduction of milk powder to these economically

disadvantaged populations. Unfortunately, there is even greater risk of malnourishment within these groups which can occur due to malabsorption of other nutrients as a consequence of diarrhoea following ingestion of milk. Providing milk under these conditions simply aggravate the problem. Lactose intolerance is also a matter of concern for emergency disaster aid and relief programs.

Means to alleviate the lactose intolerance problem: Since dairy products are popular food for many consumers, conditions for lactose intolerance are hard to avoid. What can the dairy industries and the lactose-intolerant people do to obtain a discomfort-free, yet nutritious diet?

There are several options to the lactose intolerant people regarding consumption of milk or dairy products. Lactose deficient persons may be able to tolerate varying amounts of lactose; however, it should determine one's threshold for the occurrence of symptoms associated with lactose intolerance consuming milk or dairy products containing less than known amount of lactose. The dairy industries, on the other hand, should provide information regarding the amount of lactose contained in the products so that an individual can determine the tolerance of the consumption level. Studies have shown that one's threshold for lactose tolerance may vary depending on the carrier of the lactose i.e., solid versus liquid food. Hence, whole milk should be better tolerated than skim milk. Many lactose intolerant people who show intestinal upsets to drinking moderate quantities of milk in a single dose have no difficulty in consuming the same amount of milk at intervals in smaller doses. Another option is to decrease the fresh milk consumption and increase its use in a fermented form such as yogurt. Some investigators have recommended the use of yogurt by lactose intolerant individuals as an alternative means of obtaining the nutritional benefits of milk without any discomfort. Yogurt culture organisms contain significant amount of lactase activity (Shah and Jelen, 1989) which helps the digestion and absorption of lactose from yogurt. Yogurt remains an excellent source of calcium as this fermented product is well tolerated by lactose-deficient persons. Similarly, naturally aged cheddar, Swiss and other hard cheeses can be well tolerated by lactose intolerant individuals because they are very low in lactose while containing enough calcium and other valuable dairy nutrients. However, ingestion of other fermented or microbe-containing milk such as acidophilus milk, cultured buttermilk, and kefir are not well tolerated

by lactose intolerant persons and more frequently associated with symptoms of lactose intolerance (Alm, 1982; Savaino et al., 1984).

Yet another option is to add a lactase enzyme preparation to the fluid milk prior to drinking. At present a number of food grade enzymes obtained from various microorganisms e. g., *Kluyveromyces lactis*, *K. fragilis*, *Aspergillus niger*, etc. are being marketed for home use. These enzymes hydrolyse most of the lactose making the milk more digestible for the lactose-deficient persons without any discomfort. Several brands of modified milk are available commercially for the lactose intolerant individuals; in these products, 70-90 per cent of the lactose has been converted to its monosaccharides, glucose and galactose (Anon, 1989). Thus lactose hydrolysed milk offers lactose intolerant individuals a dietary alternative to avoiding milk consumption.

However, the situation is quite different in developing nations. The commercially available enzymes are expensive. The feasibility of marketing liquid fermented product such as yogurt is limited by the shortage of refrigeration facilities. Availability of lactose hydrolysed skim milk powder for reconstitution to lactose hydrolysed milk may alleviate this problem. However, the protein quality in lactose-hydrolysed spray dried powder milk may be considerably lower due to the loss of biologically available lysine. During drying process due to employing excessive heat, the glucose, liberated from lactose by the hydrolysis, can easily react with the protein resulting in a loss of about 35-40 per cent of available lysine.

Another option is to consume milk in its fermented form. In many developing nations, home made fermented milk products similar to yogurt are very popular and are used as a part of the indigenous diet. The various international programs should encourage the lactose intolerant populations by supplying fermented milk powder to support malnutrition problems of the third world.

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BABESIOSIS IN CATTLE - A CASE REPORT

D. R. Ratala* U. M. Singh** P. Manandhar***

Summary

A causative agent of Bovine babesiosis, *Babesia bigemina* was detected in two crossbred cows and one calf belonging to a farmer from Chabahil, Kathmandu. Clinical recovery was obtained by treating these sick animals with Berenil. Recurrence of the Babesiosis was not recorded during the observation period of two months.

Case History

A farmer, resident of Chabahil, Kathmandu reported that five of his crossbred cows (three adults and two calves) are sick. Further enquiry revealed that ten adult crossbred cows along with four calves were recently imported from India. Within a few days time, two of his female calves died suddenly showing signs of anorexia, emaciation, pyrexia, jaundice and haemoglobinuria. Sick animals failed to respond to antibiotic treatment.

Clinical Findings

On clinical examination, the animals were found off feed, depressed having conjunctiva and visible mucous membrane pale. Urine was dark red in colour. Body temperature ranged from 104° F to 107° F. There were numerous ticks on the animal's body and were collected for further identification.

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Diagnosis

Peripheral blood taken from five sick animals during high fever. On examination showed severe anaemia with erythrocytic count as low as 2.5 million/cu.mm. Where as the leukocytic count was increased proportionately. Examination of the Giemsa's stained thin & thick blood smear from two cows and one calf showed a typical double, pear shaped bodies joined at the pointed end in red blood cells. About 40 per cent of erythrocytes were found parasitized. Based on morphological features and pathological characters the organism was diagnosed as *Babesia bigemina* but needs further conformation.

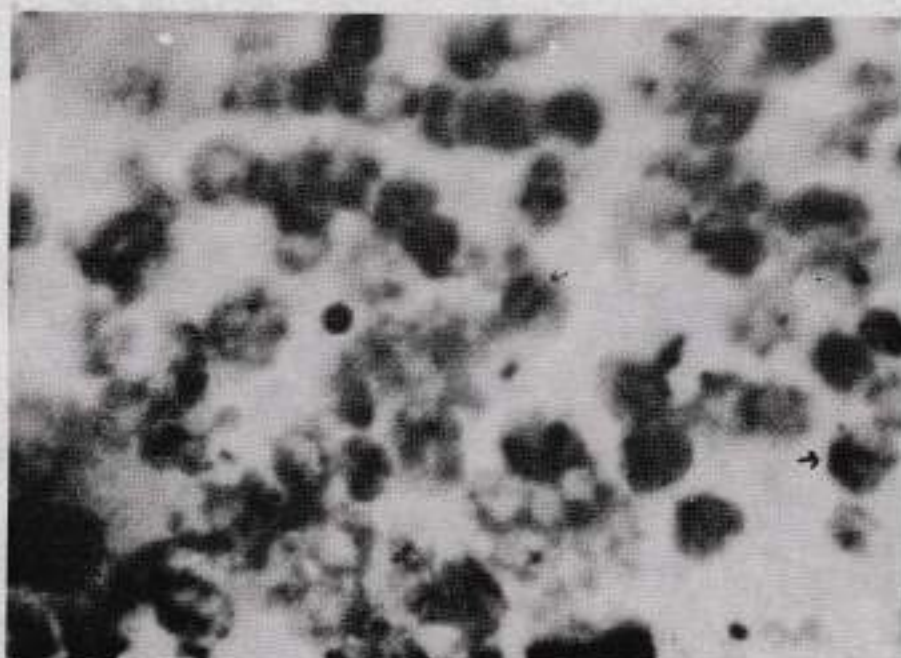
Treatment and Result

These cows were treated with Berenil R Vet. granules (Hoechst) at the dose rate of 0.8 gm/100 kg body weight by deep intramuscular injection into the neck muscles. In addition to that coumatol (pearl chemicals) was used for controlling ticks. Cal-D-Rubra (Cadila) 50 ml twice a day orally for ten days and Aminodox (E Merck) eight capsules per day for one week were also given as supportive therapy. All these therapy responded very well and clinical symptoms gradually subsided. These animals were checked regularly for two months and were found without any recurrence of *Babesia* organism in the peripheral blood.

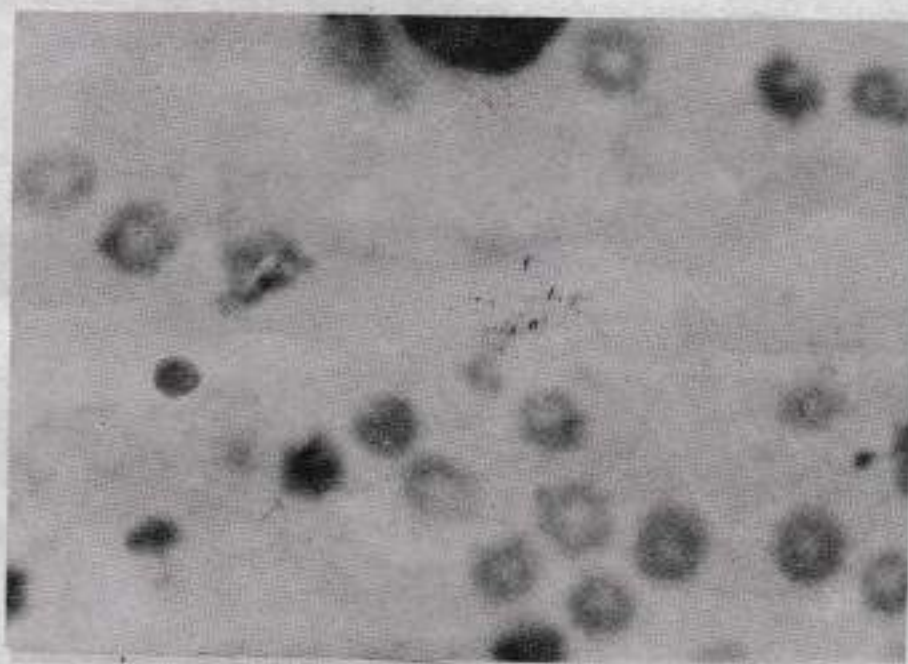
Acknowledgement

The authors are very much thankful to Dr. U. Mishra, Chief of C. D. R. L. for providing the necessary facilities for this work.





Microphotograph of the Giemsa Stained blood smear showing *Babesia nigemina* organism inside RBC.



Arrow showing the organism inside RBC of Giemse Stained blood smear in microphotograph.

SUCCESSFUL DRENCHING OF HELATAZ AND XETHANE IN ELEPHANT

Bal Ram Thapa*

Introduction

Asian elephants play a vital role in tourist industry, various national parks and wild life safari camp of Nepal. Elephant is also an endanger species. Various attempts have been made to study the diseases in elephant and their remedy. Baruah (1983) reported the common diseases of the wild life in captivity. Dekatal (1985) reported the prevalence of intestinal helminth infection among wild elephants in Assam. Borthakur et al (1988) reported a successful treatment of distomiasis in elephant with avlothane. Dutta and Bordoloi (1988) reported the incidence of intestinal helminth infection in elephant in Tiger Project, Manas, Assam. Gogoi and Dutta (1989) reported a successful treatment of ascariasis in elephant with panacure

Materials and Method

This experiment was conducted in a herd of six elephants privately owned by Tiger Top kept near Royal Bardia National Park, Thakurdar, Nepal. Out of six, three elephants were never exposed to any anthelmintics. Other three were also not drenched with anthelmintic since one year. They were lean, not willing to take food properly, gurgling sound coming from stomach but working as usual.

In one morning; drenching was done with Helataz (Parbendazole B. P. vet. four per cent w/w of SK&F) at the dose rate of 480g per elephant. Total dose was divided into ten parts and mixed in ten kuchi (concentrated food of elephant) and

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fed to elephant orally. In the same evening, at the interval of 12 hour, xethane (Hexachloroethane B. Vet. C. 85%w/w of SARABHAI) was given orally at the dose rate of 163g mixing with kuchi.

Result and Discussion

In next day morning, out of six elephants four started expelling worms along with dung. One elephant among those four was expelling flukes and worms simultaneously. One elephant showed some side effect and it suffered from tympany, off feed for about four hours. The elephant became alright in the next morning. Elephants were expelling worms and flukes for three days and then stopped subsequently. Since then all the elephants became normal and started gaining weight. Few worms and flukes were collected and preserved in ice chamber for laboratory examination. In the laboratory eggs were released by pressing the worms in between two slides and on the basis of gross and microscopical examination these intestinal helminths were grouped as follows.

Sr. No.	Name of elephant	Strongylus	Paramphistum	Gastrodiscus	Remarks
1	Chunchkali	++++	++	+	Fasciola
2	Champakali	++++	----	----	species is
3	Laxmikali	++++	----	----	not taken
4	Ganeshkali	+++	----	----	into consi-
5	Sunderkali	----	----	----	deration.
6	Madhumala	----	----	----	

Therapeutic diagnosis proved 66.6% of elephant were infested by intestinal helminths. The highest infestation recorded 66.6% was by strongylus spp. while 33.4% was free from parasite infection.

Successful drenching against worms and flukes in captive elephants can be done with commonly available anthelmintic drugs i. e. Helatac and Xethane in field condition. The present finding supports with the finding of Borthakur, Dutta & Bhattacharya (1988) about efficacy of hexachlorothane against flukes.

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MANUFACTURE OF WHEY PROTEIN CONCENTRATES (WPC) BY ULTRAFILTRATION (UF) OF CHEESE WHEY*

T. B. Thapa** and V. K. Gupta***

Conclusion

Ultrafiltration of cheddar cheese whey yielded 3 per cent whey protein concentrate of good quality showing excellent solubility in water.

Introduction

Whey is an important byproduct of cheese industry which contains more than half of the total solids of milk. About 5-9 times whey is produced per kilogram of cheese. Whey is almost a saturated lactose solution and contains highly nutritious whey proteins. Unfortunately most of this valuable byproduct is either drained off causing heavy pollution problems or utilized for non-edible purposes.

In recent years, UF technology has made it economically possible to separate whey proteins from lactose. By this technology, not only lactose is eliminated but whey proteins are also concentrated in native and wholesome state. The recovery of these highly nutritious whey proteins in the form of whey protein concentrates (WPC) and their utilization in dairy products like cheeses, processed cheese etc which would help in restoring most of the lost proteins during cheddar cheese manufacture. This would also increase the nutritive value and yield of the cheeses.

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Materials and Methods

Whey protein concentrate was manufactured by ultrafiltration of cheddar cheese whey following the method of Gupta and Reuter (1987) 500 litres of fresh cheddar cheese whey was procured from National Dairy Research Institute, Karnal and filtered through a muslin cloth. Filtered whey was clarified and separated at 30°C and pasteurized by heating to 72°C for 16 seconds. Whey was then held at $70 \pm 2^\circ\text{C}$ for 30 to 40 minutes followed by cooling to 60°C. Thereafter whey was transferred to the balance tank of UF plant. This plant consisted of Hollow Fibre membrane (Romicon, Polysulfone membrane type PM 50, fibres inside diameter-1.1mm, effective membrane area - 2.5 m² and molecular weight cut off at 50,000).

Whey was ultrafiltered at $55 \pm 2^\circ\text{C}$ using inlet pressure, 1.8 kg/cm² and outlet pressure, 0.5 kg/cm². Ultrafiltration was continued till the WPC was concentrated to about 27 per cent. The product was packed in one kilogram polyethylene bags and stored at -20°C till used.

Analytical Methods

Whey was analyzed for its chemical composition viz. Total Solid (TS), protein, and fat. Total solid was determined gravimetrically following the method of Kosikowski (1982) by heating at $100 \pm 1^\circ\text{C}$ for 6 hours. Whey was analyzed for protein as per the method of El-Sokkary *et al* (1957) following Semimicrokjeldahl method of Menefee and Overman (1940). Fat was determined by Gerber method using skim milk butyrometer. WPC was analyzed for total solids, fat, protein, total ash, calcium, titratable acidity and pH.

Total solids were determined gravimetrically by drying at $100 \pm 1^\circ\text{C}$ for 10 hours as described by Kosikowski (1982). Fat was determined after making 10 times dilution by Gerber method using skim milk butyrometer. Protein was determined as per the method followed in whey analysis by weighing 200 mg of sample. Total ash was determined by A. O. A. C. (1980) method by igniting at $550 \pm 20^\circ\text{C}$ for 5 hours. Calcium content and titratable acidity was calculated as per the method described in SP:18 (part - XI) - 1981. The pH was recorded at 20°C on pH meter (ELTOP 3030) using single glass electrode.

Results

Ultrafiltration of whey (8.33 per cent TS, 0.857 per cent Protein and 0.05 per cent Fat) till it attained 27.41 per cent TS yielded 15 kilograms of whey protein concentrate. Whey protein concentrates thus manufactured had following composition:

S.N.	Parameter	Per cent
1	Total Solids (T. S.)	27.41
2	Fat, on dry basis	9.12
3	Protein, on dry basis	71.11
4	Total ash, on dry basis	3.313
5	Calcium, on dry basis, (mg%)	267.85
6	Lactose (by minus method)	16.45
7	Acidity (% LA)	0.598
8	pH	6.22

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HIMALAYAN CHERRY POISONING IN SHEEP: A CASE REPORT

S. N. Mahato*

Introduction

Himalayan cherry (*Prunus cerasoides*), locally known as *Paiyun* is a common tree species growing between the elevation of about 900–2400 m in the hills of Eastern Nepal. Although feed quality of the leaves is considered to be poor, their contribution as a green supplement to ruminant nutrition is appreciable, especially at a time when other fodder leaves are scarce. In recognition of the importance of this plant and other species of tree crops in the area, an investigation to evaluate the fodder quality of different tree species has been undertaken at the Pakhribas Agricultural Centre, Dhankuta. The case reported herein was an incidental finding during the course of investigation.

Case History

During second week of April 1987, two sheep and two goats were kept on a full diet of Himalayan cherry (*Paiyun*) leaves in order to estimate their DOMD, DMD and OMD content. The mean voluntary intake by the animals was 64, 72 and 80 g of fresh leaves (43.8 % drymatter) per kg body weight on first, second and third day of feeding, respectively. On the evening of the third day staggering, muscle tremor and dyspnoea was observed in one of the sheep which had highest intake of the leaves (94 g per kg body weight) that day. The sheep was found dead on the following morning. There was profuse exudation of bright red blood

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from the nostrils.

Autopsy revealed dark colouration of the skeletal muscles, blood stained peritoneal fluid, congestion and haemorrhages in the trachea and lungs, subpericardial and endocardial haemorrhages, and distension of the veins.

The clinical and the autopsy findings suggested hydrocyanic acid poisoning which was confirmed by testing the rumen fluid for the presence of cyanide according to the method described in manual of Veterinary Investigation Laboratory Techniques (Anon, 1981).

Discussion

The leaves of other species of cherry such as *Prunus amygdalus*, *Prunus demissa* and *Prunus laurocerasus* contain cyanogenetic glucocides, amygdalin and prucetin (Blood, Radostits and Henderson, 1983; Singh, R. S., 1983). It is known that hydrocyanic acid poisoning may result if large quantities of the leaves of *P. demissa* and *P. laurocerasus* are eaten (Blood, Radostits and Henderson, 1983). However there appears to be no record of hydrocyanic acid poisoning in animals due to ingestion of *Prunus cerasoides* leaves. It has been mentioned in Medicinal Plants of Nepal (HMG/N Publication) that smaller branches of *P. cerasoides* are used as substitute for hydrocyanic acid. The present findings suggest that the leaves of *P. cerasoides* also contain cyanogenetic substances and ingestion of large quantity of the leaves may result toxicity and fatal to animals.

It is interesting to note that farmers in Nepal are already familiar with some of the ill-effects of this species on ruminants such as urinary problems and decrease in milk yield (Panday, 1982). They believe that the toxic substances responsible for these ill effects are high in young leaves and remain in the leaves only until the onset of premonsoon rain. Thus use of this species as fodder is rare before this period. Furthermore, farmers are careful not to give more than 25 per cent of this plant in their animals diet. Therefore it seems that the lethal poisoning is avoided by farmers by restricting the quantity included in the animals diet and by avoiding its use as fodder during the period when the cyanogenetic glucocide content of the leaves is highest.

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EFFECT OF DIFFERENT TREE FODDERS ON MILK PRODUCTION IN BUFFALOES

N. P. Shrestha*, B. Pakhrin**

Summary

Lactating buffaloes on farms were given one of six different tree fodders, Tanki (*Bauhinia purpurea*), Nebharo (*Ficus auriculata*), Kutmero (*Litsea monopetala*), Ryekhanyu (*Ficus semicordata*), Khasrekhanu (*Ficus cunia*) and Shyalphusro (*Grewia oppositifolia*) for a period of two weeks. For all fodders except Nebharo milk yield increased above the initial value by 0.2 to 0.5 kg per day when the tree fodders was fed. The reasons for the decreased milk yield resulting from feeding Nebharo are thought to be due to its poor digestibility and the presence of high amounts of hemicellulose and tannins.

Introduction

The growing of fodder trees is an old, well known traditional practice in the villages of Nepal. Trees are one of the major sources of green fodder for ruminants in the winter season when there is little forage available. Pandey (1982) estimated that in the hilly areas 35 per cent of the total feed for all farm animals is supplied by the fodders. Hopkins (1985) has also examined the importance of tree fodder in Nepal. Gatenby et al (1989) reported that 60 per cent of the feed given to buffaloes in the Koshi Hills is tree fodder.

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This experiment was carried out to find out the feeding value of various tree fodders for lactating buffaloes during the dry season.

Materials and Methods

The experiment was conducted on household farms in the four Panchayats of Dhankuta district. The six species of fodder trees namely, Ryekhanyu (*Ficus semicordata*), Khasrekhanyu (*Ficus cunia*), Tanki (*Bauhinia purpurea*), Shyalphusro (*Grewia oppositifolia*), Nebharo (*Ficus auriculata*), Kutmere (*Litsea monopetala*) were included because they were popular and were available in sufficient amounts during the winter season. Each tree fodder was fed to two lactating buffaloes except Tanki which was fed to six buffaloes.

The duration of the experiment was one month. During the whole period of the experiment each buffalo was given 10 kg rice straw plus 3.4 kg of a locally prepared concentrate ration per day. In the first week of the experiment no tree fodder was fed. In the second and third weeks *ad libitum* tree fodder was given.

Fodder tree samples were collected during the second and third weeks of the experiment and were analysed in the laboratory. Proximate analysis were conducted for all samples, but digestibility, hemicellulose content and tannin content were determined for only Nebharo and Ryekhanyu. Milk yield and feed intake were measured twice daily, in the morning and evening.

Results

Analysis of fodders

The composition of fodder samples was similar in week two and week three. The mean values are given in Table 1.

Table - 1 : Analysis of tree fodder.

Species	DM %	Dig %	CP %	NFE %	EE %	CF %	TA %	H %	T %
Nebharo	39	29.6	10.9	59.1	2.3	13.6	14.1	21.0	2.6
Ryekhanyu	38	54.1	11.2	59.3	1.0	14.0	13.6	6.5	6.2
Khh. Khanyu	41	-	9.9	59.7	2.1	16.2	17.0	-	-
Shyalphusro	46	-	11.6	50.8	1.6	24.6	11.4	-	-
Tanki	43	-	12.6	46.3	2.4	28.0	10.7	-	-
Kutmero	43	-	8.2	58.2	2.2	22.5	8.9	-	-

(DM = Dry matter, Dig = Digestibility, CP = Crude protein, NFE = Nitrogen free extract, EE = Ether extract, CF = Crude fibre, TA = Total ash, H = Hemicellulose and T = Tannin).

The dry matter per centage of the tree fodders ranged from 38 to 46 per cent. These values are considerably higher than those for green grass or green legumes which are typically 18-20 per cent, but are much lower than values for dry foods such as straw which are about 86 per cent (McDonald 1981). Moderate levels of dry matter encourage maximum food intake.

The crude protein levels range from 8.2 per cent for Kutmero to 12.6 per cent for Tanki. These values are reasonable for ruminants. It is surprising that the crude protein levels measured here are all considerably lower than those cited by Panday (1982). For example, Panday cites CP levels of 17.3 per cent for Nebharo and 29.7 per cent for Tanki.

The digestibility of Nebharo (29.6 per cent) was much lower than that of Ryekhanyu (54.1 per cent) The higher hemicellulose content of Nebharo may partly account for this difference. Both species were found to contain high levels of tannins.

Milk yield

Figure 1 shows the average milk yield for each group of buffaloes during the

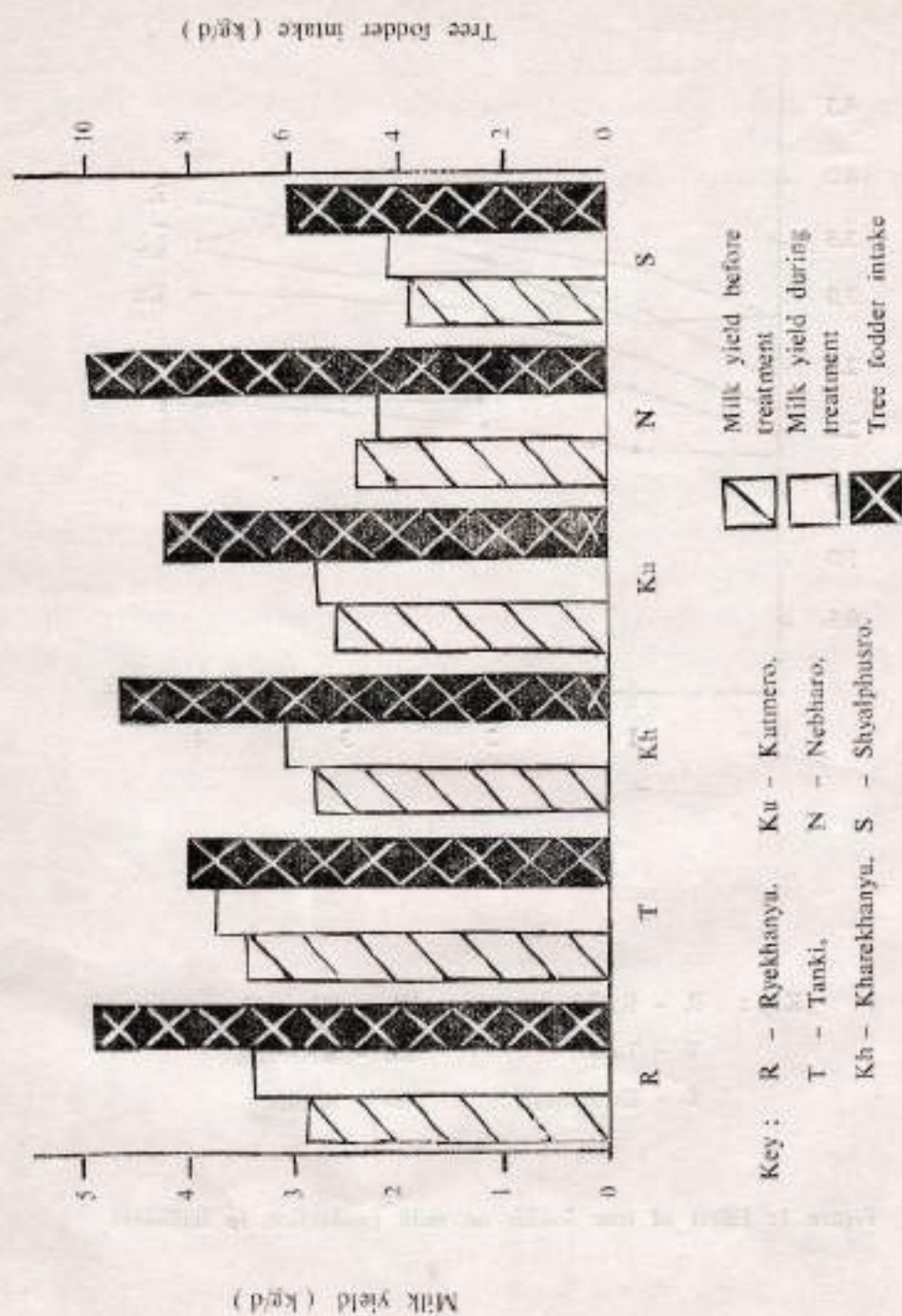


Figure 2: Tree fodder intake and milk yield before and during treatment.

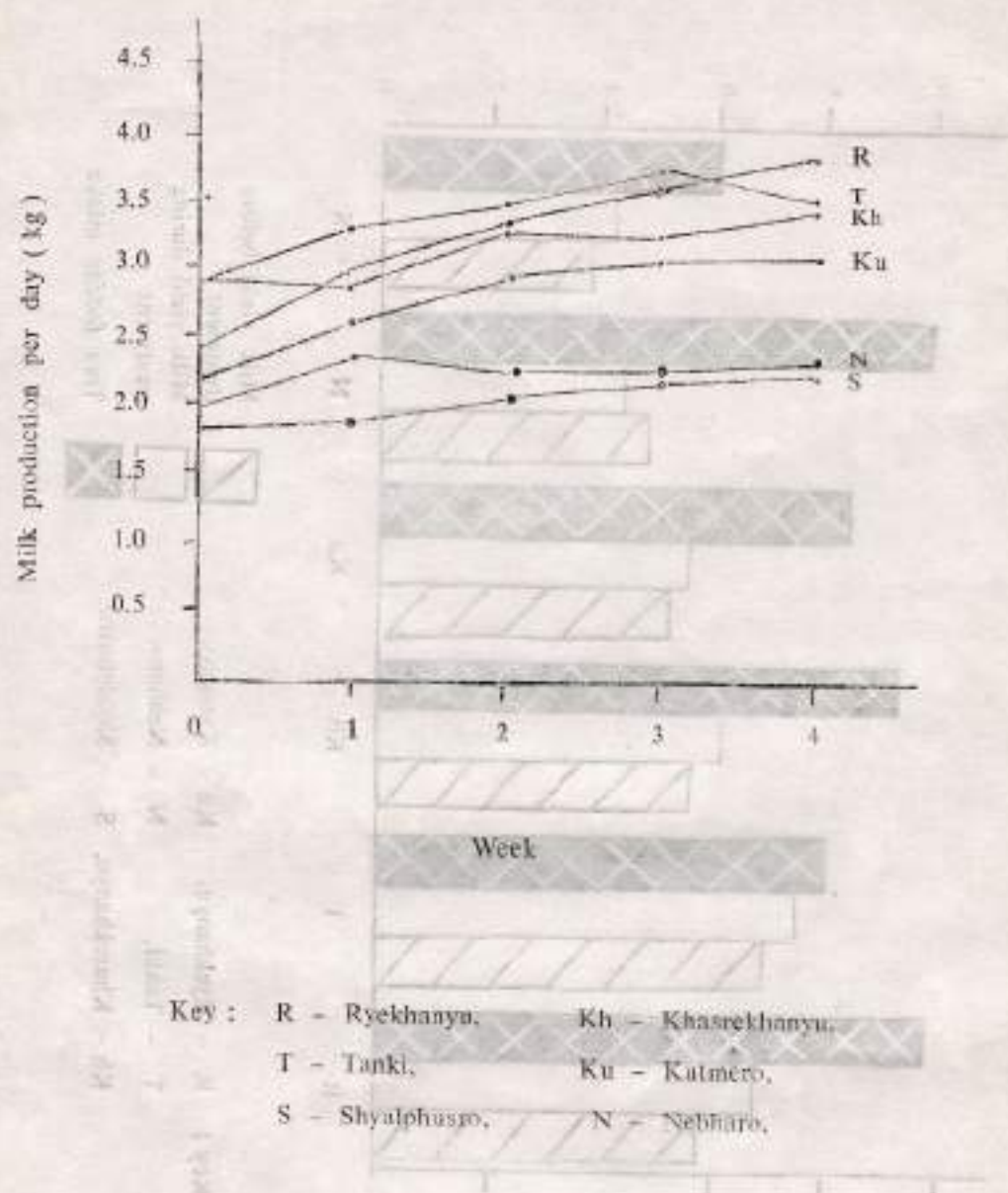


Figure 1: Effect of tree fodder on milk production in buffaloes.

experiment. The figures for week zero are those obtained on day zero of the experiment. The others are the averages for each week.

In general when cows are on a constant diet, milk yield declines gradually as lactation proceeds. However, for all except one group of buffaloes, average milk yield in the first week was higher than that before the start of the experiment. This indicates that the diet during week one was better than the normal diet provided by the farmers.

The milk yield in weeks two and three when the fodder trees were fed were in general higher than the yield in week one. The increase in milk yield over the first week values gives an indication of how effective the tree fodder is in stimulating milk yield.

The average increases in milk yield as a result of feeding tree fodders are shown in Figure 2. Milk yield increased by 0.2 to 0.5 kg per day when Ryekhanyu, Khasrekhanyu, Tanki, Kutmero, and Shyalphusro were fed but decreased by 0.2 kg per day when Nebharo was fed.

Analysis of variance indicates no general effect of the type of tree fodders on milk yield at ($p < 0.05$). However, comparison between individual means in Table 2 showed that the cow given Nebharo responded negatively from those other fodders.

Table - 2 : Mean value and standard deviation of increase in milk yield and tree fodders intake.

	Tanki	Kutmero	Shyal- phusro	Nebharo	Khasre- khanyu	Ryekhanyu
Increase in milk yield (kg)	0.34+0.15	0.25+0.33	0.23+0.05	-0.21+0.01	0.36+0.31	0.51+0.51
Tree fodders intake (kg)	8.05+1.7	8.5+0.14	6.07+0.60	10.0+0	9.4+0.30	9.85+0

In week four, when the buffaloes were no longer given fodders milk yield stayed above the first week value, except for the buffaloes which were given Nebharo and Tanki. This suggests that there is a positive residual effect of certain tree fodders on milk yield.

Tree Fodder Intake

Figure 2 shows that the intake of tree fodders ranged from 6 kg per day for Nebharo. Analysis of variance showed that in general the type of tree fodders affected intake at ($p < 0.05$). However, Table 2 showed that the intake of Shyalphusro was significantly lower than the intake of the other tree fodders. It is interesting that even though Nebharo had a negative effect on milk yield, the intake of Nebharo was higher than the intake of other tree fodders.

Discussion

It is generally believed that tree fodders boost the milk yield of lactating animals. Figure 1 has shown that there was an increase in milk yield when tree fodder was added to the diet of buffaloes.

All fodders except Nebharo had similar effects. The animals given Nebharo responded with a lower milk yield. It may be due to its poor digestibility, or high content of hemi-cellulose and tannin. A decrease in milk yield from Nebharo has also been reported by Panday (1982), Anderson (1947) and Allan (1974). They have also reported toxicity resulted to livestock from feeding various tree fodders and shrubs. Toxicity varies with environmental conditions, stage of growth, amount fed and condition of the stock.

Further Elaborate

Nebharo is considered to be one of the most popular fodder tree with high nutritive value, and is widely planted in the hills of Nepal. The same tree fodder in a mixture with other plants may not adversely affect milk yield. Combinations of other fodder trees with Nebharo may therefore have positive effect. Research to investigate this effect is certainly worthwhile.

It must be remembered that only two animals in this experiment were given Nebharo due to the constraint of finding large numbers of uniform animals on farms. Before any recommendations can be made further experiment with more numbers of animals is needed to confirm this negative effect.

Acknowledgement

We thank Shreeram Neopane for help in conducting this experiment, Dr. S. N. Mahato and colleagues for analysing the fodder samples, and Ruth Gatenby and other colleagues in the Livestock Section for assistance with the analysis of the data and the preparation of this report.

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STORAGE STUDY OF PROCESSED CHEESE FOODS AND PROCESSED CHEESE, ITS EFFECTS ON RHEOLOGICAL CHARACTERISTICS*

T. B. Thapa** and V. K. Gupta***

Abstract

Rheological measurements were carried out in the processed cheese food and processed cheese samples stored at $37 \pm 1^\circ\text{C}$. During storage, hardness increased, cohesiveness and springiness mostly decreased, and adhesiveness, gumminess and chewiness showed varying trend in processed cheese foods and processed cheese samples.

Summary and Conclusion

With the advance of storage period, processed cheese foods/processed cheese samples showed progressive increase in hardness. Cohesiveness mostly decreased, but no conclusive effect of added WPC was observed in cohesiveness changes during storage. It was observed that a 3.33 to 23.07 per cent decrease in springiness values in stored samples. Adhesiveness, gumminess and chewiness showed a varying trend in stored PCFS and processed cheese samples. Experimental processed cheese showed 44 per cent increase in adhesiveness and slight decrease in gumminess during 83 days of storage. Slight decrease in chewiness was observed in almost all samples except PCF (20.25 per cent).

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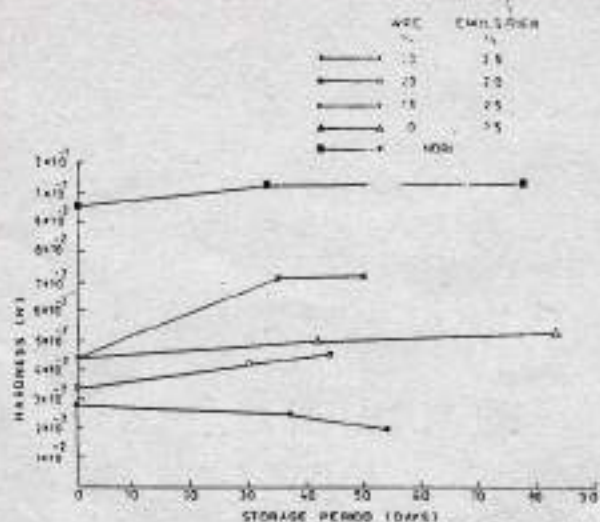


FIG 2-1 CHANGES IN HARDNESS OF PCFs/PCs DURING STORAGE

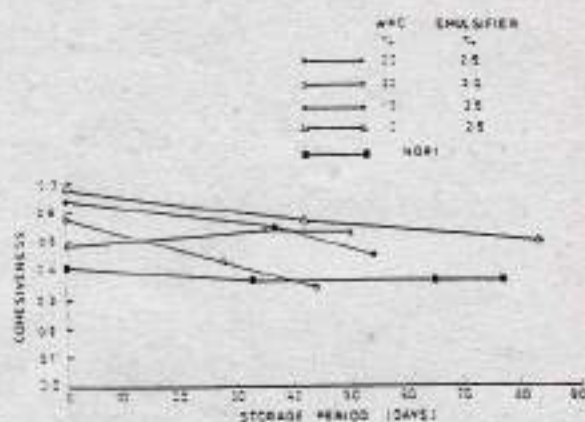


FIG 2-2 CHANGES IN COHESIVENESS OF PCFs/PCs DURING STORAGE

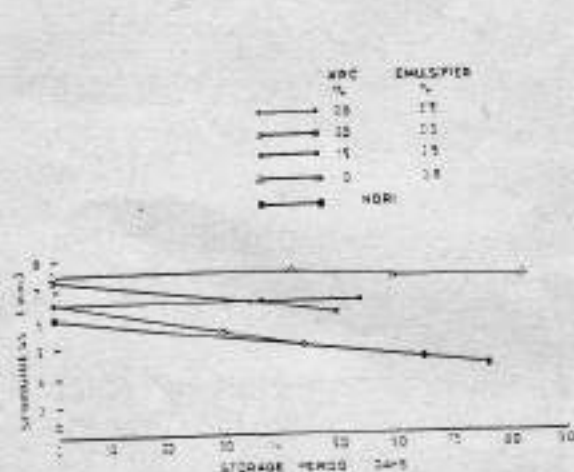


FIG 2-3 CHANGES IN SPRINGINESS OF PCFs/PCs DURING STORAGE

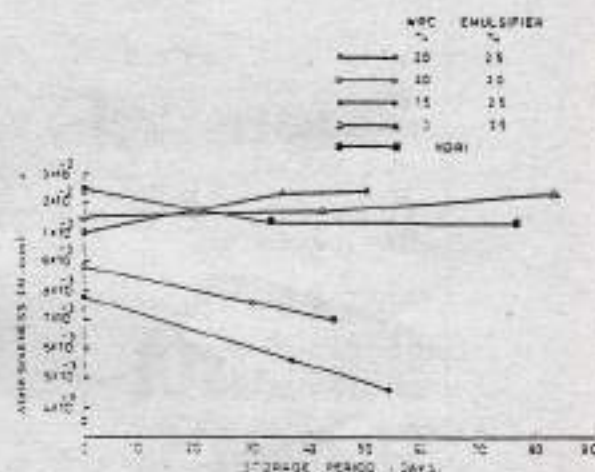


FIG 2-4 CHANGES IN ADHESIVENESS OF PCFs/PCs DURING STORAGE

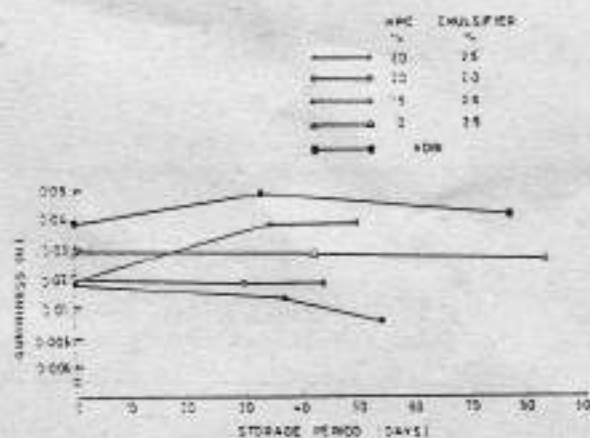


FIG 2-5 CHANGES IN GUMMINESS OF PCFs/PCs DURING STORAGE

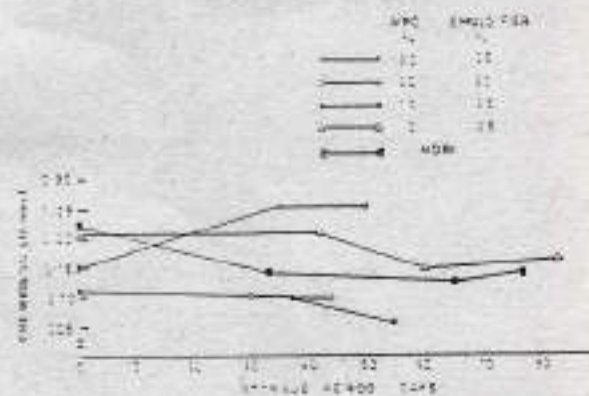


FIG 2-6 CHANGES IN CHEWINESS OF PCFs/PCs DURING STORAGE

Introduction

Quite good amount of processed cheese food (PCF) is prepared in USA, USSR and many East European countries, but many standardization works on this product are still to be carried out. There is no published work on storage quality of processed cheese foods with added whey protein concentrates (WPC) under our condition, and more particularly on changes in rheological characteristics during storage. There exists a relation between sensory acceptability and rheological characteristics, thus changes in rheological measures indirectly affects the sensory acceptability of products. Hence, this investigation was undertaken.

Materials and Methods

Kosikowski (1982) method with some modifications was used for manufacturing processed cheese food and processed cheese.

Whey protein concentrate was manufactured by ultrafiltration of Cheddar Cheese whey following the method of Gupta and Reuter (1987).

Processed cheese food/processed cheese tins were stored in an incubator maintained at $37 \pm 1^\circ\text{C}$ and evaluated for changes in rheological characteristics during storage.

Cheese samples were measured for their rheological characteristics like hardness, cohesiveness, springiness, adhesiveness, gumminess and chewiness using an Instron Universal Testing Machine (Model 4301, Instron Corporation, Canton, U.S.A.). The machine was fitted with a reversible load cell (100 Newtons) and was operated with a cross-head speed of 20 mm/min, a chart recorder speed of 40 mm/min (cross-head recorder ratio of 1:2) and recorder range of 10 per cent (full scale chart deflection of 10 Newtons).

For Instron analysis, processed cheese foods/processed cheese samples (2.1 cm dia. and 2 cm height) were drawn out using cork borer and a sharp razor blade. Each sample was compressed twice to 80 per cent of its height (i. e. to 1.6 cm). A typical force distance profile obtained from the Instron texture profile analysis (TPA) is illustrated in Fig. 1.

Textural variables were calculated according to procedure of Gupta *et al.* (1984), which were as follows:

- i) Hardness (in N) is the height of first peak during the first bite.
- ii) Cohesiveness is the ratio of area under second peak to first peak or A_2/A_1 .
- iii) Springiness (mm) is the distance, the sample is under compression during the second bite.
- iv) Adhesiveness (N-mm) is the negative force area for the first bite (A_3).
- v) Gumminess (N) is hardness $\times$ cohesiveness.
- vi) Chewiness (N-mm) is gumminess $\times$ springiness.

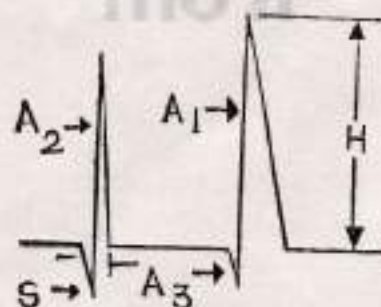
Results and Discussion

Processed cheese foods (PDFs) conforming to U. S. Standards (43.32 - 43.56 per cent moisture), experimental processed cheese having moisture 41.76 per cent, and NDRI processed cheese were stored at $37 \pm 1^\circ\text{C}$ and studied for changes in rheological characteristics. Results so obtained are discussed below:

Changes in Rheological Characteristics

Hardness: Fig. 2.1 illustrates the changes hardness of PCFs and processed cheese samples during storage. With the advance of storage period, PCFs (20 per cent) and processed cheese samples showed progressive increase in hardness except PCF (15 per cent), which depicted a slight but gradual decrease in hardness during storage. Leung *et al.* (1976) reported increase in hardness of PGF stored for 4 months at room temperature. Sherman (1976) also observed increase in hardness, viscosity and elasticity of processed cheese stored at 5°C for 2 months. Increase in hardness may partially be due to decline of pH during storage. Olson *et al.* (1961) reported that decreasing pH causes marked increase in firmness of cheese spreads during storage at 90°F .

Cohesiveness: Fig. 2.2 compares the cohesiveness of PCFs with processed cheese samples after different interval of storage period. In general, most of the stored PCF and processed cheese samples showed slight decrease in cohesiveness except PCF (20, 2.5 per cent), which depicted a very slight increase in cohesiveness with



H - HARDNESS

A_2 / A_1 - COHESIVENESS

A_3 - ADHESIVENESS

S - SPRINGINESS

FIG. 1: A TYPICAL FORCE DISTANCE PROFILE OBTAINED FROM THE INSTRON TEXTURE PROFILE ANALYSIS.

storage. No conclusive effect of added WPC was observed in cohesiveness changes during storage.

Springiness: Fig. 2.3 illustrates the effect of storage period on springiness of PCFs and Processed cheese samples. In general, stored PCF and processed cheese samples showed decrease (3.33 to 23.07 per cent) in springiness values during storage.

Adhesiveness: Fig. 2.4 describes the changes in adhesiveness values of PCFs and processed cheese samples during storage. PCFs showed decline in adhesiveness (19.5 - 40 per cent) during storage except PCF (20, 2.5 per cent), which showed almost two-fold increase. Experimental processed cheese (2.5 per cent) showed 44 per cent increase in adhesiveness during 83 days of storage. NDRI processed cheese, however, showed a decrease of 41.6 per cent in adhesiveness during storage.

Gumminess: Fig. 2.5 illustrates the gumminess of PCFs and processed cheese samples during storage. Varying trends in gumminess changes were observed during storage period. PCF (20, per cent) showed no changes in gumminess, but PCF (20, 2.5 per cent) and PCF (15, 2.5 per cent) respectively, showed increasing and decreasing trend during storage. Experimental processed cheese (2.5 per cent) showed very slight decrease in gumminess during 83 days of storage. NDRI processed cheese had a very slight increase in gumminess.

Chewiness: Fig. 2.6 illustrates the changes in chewiness of PCFs and processed cheese samples during storage. PCFs did not show any definite trend in chewiness changes during storage. All samples of PCFs and processed cheese showed slight decrease in chewiness during storage with the exception of PCF (20, 2.5 per cent), which showed considerable increase in chewiness.

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A BRIEF INTRODUCTION TO BIOLOGICAL PRODUCTS DIVISION

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History

Biological Products Division (BPD), the only institution so far involved in production, testing, standardization and distribution of veterinary vaccines against the commonly encountered diseases of livestock and birds in the country is located in the Veterinary Complex, Tripureswor, Kathmandu, Nepal. Originally the activity was initiated in 1953 with the use of wet Goat Tissue Rinderpest Vaccine in order to control the devastating outbreak of Rinderpest disease in Pokhara valley. In 1960, Veterinary Laboratory was established under the financial and technical cooperation of Indian Government, Indian Cooperative Mission (ICM) and was further assisted technically and financially by FAO, OXFAM, ADB, and HMG/N. Biological Products Division (BPD) was established in 1971 as an independent identity / laboratory and from 1983/84 it is functioning under the revised organization of Central Animal Health Centre of the Department of Livestock Services, Ministry of Agriculture, HMG Nepal.

Objectives

The main objective of this division is to produce, standardize, and supply of various veterinary vaccines against the most common and economically important diseases of animals and birds prevalent in Nepal. The division is trying to improve the quality as well as quantity of its present products and also aiming to introduce some more vaccines such as Marek's disease vaccine, Rinderpest Tissue Culture

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Vaccine, Swinefever vaccine, antiserum and antiserum for diagnostic purposes in the near future. At present the division is able to meet the national demand of vaccines against Black-Quarter, Haemorrhagic Septicaemia, Anthrax, Rinderpest, Fowl Pox and Newcastle diseases of animals and birds.

Activities

The division is operating at four major vaccine production laboratories viz. Bacterial, Tissue Culture, Poultry and Quality Control together with some other supportive units such as Laboratory Animal, Supply, Administration, Account and Maintenance. At present, out of 57 total posts sanctioned, 32 technical and 14 nontechnical personnels are working in this division. During the fiscal year 2045/46 (1988/89) a total of 6937600 doses of different vaccines against different animals and birds diseases were produced by this division and all of them were distributed free of cost through-out the country through governmental or non-governmental organizations, farms or personnel as and when required in order to prevent or control the diseases. These vaccines are also used to meet the vaccination target of the Government. Total vaccines produced by this division are as follows:

Name of vaccines	Annual production (2044/45) 1987-88 Doses
A. Viral Vaccines:	
1. Rinderpest Goat Tissue Vaccine	8 lakh
2. Newcastle Disease F-1 strain Vaccine	20.4 lakh
3. Newcastle Disease M- Vaccine	18.33 lakh
4. Fowl Pox Vaccines	10.23 lakh
B. Bacterial Vaccines:	
1. Haemorrhagic Septicaemia Oil Adjuvant Vaccine	0.72 lakh
2. Haemorrhagic Septicaemia Alum Adjuvant Vaccine	7.60 lakh
3. Black Quarter Alum Adjuvant Vaccine	2.0 lakh
4. Anthrax Spore Vaccine	0.48 lakh

Rinderpest Goat Tissue Vaccine, Haemorrhagic Septicaemia Plain Broth Vaccine, Newcastle Disease Vaccine, Fowl Pox Vaccine and Rabies (sheep brain) vaccines were produced during the year 1965/66, 1967, 1968, and 1970 respectively in this division. However, Rabies vaccine is being produced separately by Rabies Control Project since 1981/82 and rest of the vaccines have been continuously produced by BPD. BPD is trying to improve quality of its existing products incorporating new essential biologicals in every successive periods by applying modern technology as far as possible. Since the year 1989, all of the bacterial vaccines, except Anthrax, are being produced by the "Fermentor Technique" - where 800 litres of a vaccine can be produced at a time. The establishment and utilization of large Freeze Driers for lyophilization of viral vaccines has made a significant contribution in increasing the quality as well as quantity of viral vaccines. The renovation of laboratories and the provision of some essential physical facilities helped in the achievement of targets of this division. Furthermore, BPD could successfully replace the plain bacterin of Black Quarter vaccine produced in the year 1980 by the introduction of Alum Adjuvant Vaccine this year. Similarly, Haemorrhagic Septicaemia Oil Adjuvant Vaccine - which is one of the best vaccine intended to produce active immunity for a longer period as compared to other vaccines in ruminants and the Anthrax Spore Vaccine (living) have also been produced, tested and used with very satisfactory result for the first time in Nepal in the year 1989.

The establishment of Tissue Culture Laboratory and the production of cell cultures from lamb kidneys, guinea pig kidneys and some other established cell lines further opened a new door of BPD especially in the field of production of tissue culture vaccines and newer biologicals within the country. A trial batch of Rinderpest tissue culture vaccine has already been produced and some more progress has also been made to produce this vaccine in large quantity.

Discussion

Some of the old biologicals which are not in use have been now replaced by modern biologicals. Academic support to the laboratory personnel through various training and higher education in their relevant fields would be much helpful for improving the quality of existing biologicals and practicing the modern technology

replacing the traditional methods of biologicals production. Maintenance of cold chain for vaccine storage has always been a serious problem in the field and occasional outbreak of diseases among vaccinated herd/flocks have been noticed in the country. Hence the laboratory has to be facilitated for the production of heat resistant viral vaccines especially in the production of Newcastle disease and Rinderpest disease vaccines so that the burning problem of the country in this field could be solved. However, some progress has been made in the production of Oral vaccine of Newcastle disease in poultry.

Production of biologicals is a very difficult task in existing situation with many constraints such as lack of trained and qualified man power, lack of incentives or facilities to them, non availability of essential quality materials and sufficient budgetary support. BPD is firstly trying to support the country to become self sufficient in veterinary biologicals alongwith playing a vital role in saving huge amount of foreign currency secondly safeguarding the national animal wealth protecting their health by immunization or prompt control of dreadful diseases during outbreaks. Which ultimately lead to increase the production and productivity of livestock and birds in strengthening the national economy. It is very difficult to evaluate the impact of BPD in terms of monetary value, however its role in protecting national animal wealth and there by fulfilling the basic needs of Nepalese people could be considered significant.

