

**Indian Society
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NUCLEAR RESEARCH LABORATORY

INDIAN AGRICULTURAL RESEARCH INSTITUTE NEW DELHI-110012



SYSTEMS

DATE FROM

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ABSTRACTS

NATIONAL SYMPOSIA

on

Indirect Effects of Radiation in
Relation to Food Preservation

and

Protein Metabolism in Ruminants

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NUCLEAR RESEARCH LABORATORY
Indian Agricultural Research Institute
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Session I : Indirect Effects of Radiation in
Relation to Food Preservation:

Chairman : Dr. M.S. Swaminathan, F.R.S.

SOME EFFECTS OF FEEDING IRRADIATED
WHEAT

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The inclusion of freshly irradiated wheat into diets of mice and rats was found to be associated with an increase in the frequency of polyploid cells in their bone marrow. Similarly, in monkeys and children, feeding of irradiated wheat was associated with the appearance of abnormal cells following the culture of peripheral lymphocytes. No such changes were seen when either unirradiated wheat or irradiated wheat stored for a period of 12 weeks was incorporated into diets.

Rats fed diets containing freshly irradiated wheat, but not stored irradiated wheat, were found to have lower number of circulating T lymphocytes, reduced number of antibody producing splenocytes and lower titres of antibodies following a challenge with four different antigens. These data indicate that the Immune status of these animals was somewhat impaired.

It is suggested that it would be prudent to store irradiated wheat for a period of at least 12 weeks before it is made available for human consumption.

STUDIES ON THE MUTAGENIC AND CYTOGENETIC
EFFECTS OF IRRADIATED WHEAT IN MICE

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A series of experiments were conducted to test the mutagenic and cytogenetic potentials of freshly and stored irradiated wheat in mice. In the first series, the effects of feeding of mice for various periods with the diet containing 60% of wheat irradiated with 20 and 200 Krad gamma rays were studied. The results obtained from dominant lethal tests, specific locus mutation test, studies on chromosome rearrangements in spermatogonia and gonadal cell survival indicated no positive evidence for subtle genetic and cytogenetic effects. In the second series, the studies on the effects of feeding of irradiated (75 Krad) wheat to C3H/He mice were undertaken. Feeding of both freshly and stored irradiated wheat showed neither an increase in dominant lethals and chromosomal aberrations nor a reduction in germ cells. In another series, the reproductive performance of the females fed stored irradiated (75 Krad) wheat was investigated and it was observed that the total number of litters and the litter size did not vary from those of the females fed unirradiated wheat.

MICROBIOLOGICAL ASPECTS OF STORAGE OF
IRRADIATED POTATOES AND WHEAT

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Radiation provides an effective means for extending the post-harvest storage life of perishable foods, by eradication of microbial and insect pests and by controlling physiological processes such as sprouting, ripening etc. Based on extensive studies over the past thirty years in India and abroad, WHO have recently recognised potatoes and wheat as 'unconditionally' safe for human consumption along with several other items of food.

In potatoes exposed to 10 Krad of gamma-irradiation sprouting and tuber moth infestation were completely eliminated, irrespective of the storage temperature. The ambient temperatures could not however control the microbial rotting of the tubers. Hence, irradiation followed by cool storage (10 - 15°C) offers an economical method alternative to storage at 0°C. Microbiological studies have indicated that spoilage at temperatures above 15°C are due to Erwinia spp. while dry rotting below 10°C could be attributed to Micrococcus spp. The tubers were not amenable to fungal attack by organisms of Aspergillus spp. during storage due to phenolic compounds which were otherwise labile to heating.

The total microbial population, particularly mold count was reduced by 1-2 log cycles by gamma-irradiation of wheat at 30 Krad, while fumigation methods were ineffective in controlling the mold growth during 6 months storage. The studies with artificial loading of grains with conidia of Aspergillus flavus showed a correlation between growth and relative humidity (RH) which determined the moisture content in the grain. Aflatoxin production could occur only above a critical level (13%) of grain moisture at RH value over 90% at 28°C ± 2°C. The toxin produced in irradiated grains seemed to be lower than in the unirradiated controls, indicating that radiation treatment did not cause any alterations in wheat which could otherwise promote aflatoxin producing potential.

VARIATION IN THE PROTEIN PROFILES IN THE
GAMMA-IRRADIATED CHICK PEA (Cicer arietinum L.)
SEEDS

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Water soluble seed proteins from the control as well as gamma ray treated material from the M₂ generation of Bengal gram (Cicer arietinum L.) were separated by disc electrophoresis using 7% acrylamide gels. Average Rf values and percentage of similarity values were calculated. The comparisons of number and Rf values of protein bands were made to elucidate the differences in the treated material. Differences obtained in the seed protein profiles of the treated material suggests the presence of the qualitative variation in the proteins. There were eight bands in the gels of the control sample, whereas in some of the mutants nine bands could be noticed. This suggests the presence of a new protein. In some other mutants there was a loss of one or more bands, corresponding to the loss of that particular protein. However, present studies indicate that the increase or decrease in the number of protein bands could not be correlated with a little increase or decrease in the total protein content in the seeds.

PROSPECT OF ISOLATING TOXIN FREE CULTURES
ASSOCIATED WITH HIGHER PROTEIN CONTENT IN
L. SATIVUS L.

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In two recent reports (Prasad & Das 1977 and Das & Prasad 1977), the effect of 10, 20, 30, 40, and 50 kR of gamma-rays on BOM and protein contents in six different varieties of Lathyrus sativus L. have been described.

The purpose of the present report is to compare the relative content of BOM and protein in M₂ generation in Lathyrus sativus L. (Varieties, P₁₀, P₂₄, P₂₈₈, P₂₉₃, P₅₈₅ and LC 76). It could be discerned that no correlation existed between BOM and protein content, although dose effect was apparent. However, lowering in BOM alongwith increase in total protein was noticed only at 30 kR in varieties P₁₀ and P₂₄. These results emphasise that the path of synthesis of BOM and protein is independent of each other and thereby the potentialities of an isolation of toxin free mutants coupled with increase in protein content.

Session II : Protein Metabolism in Ruminants

Chairman : Dr. B.K. Soni.

LATEST RESEARCH FINDINGS ON PROTEIN
METABOLISM IN RUMINANTS

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The atmospheric nitrogen is fixed by the soil bacteria to be utilized later by plants to synthesize proteins. These plant proteins are fed to livestock to meet their requirements for growth and milk production etc. Rumen microbes further synthesize proteins of higher biological value which are utilized by the host animal. Conversion to microbial protein in the rumen depends upon the solubility of available proteins from dietary source. Suitable pH (6.5), ammonia level and availability of sugars in rumen are very favourable factors for microbial protein synthesis. Even adenine, guanine, hypoxanthine, xanthine, uric acid and thymine are deaminated to contribute some ammonia to the general pool. Quality of protein of dietary origin is important because different proteins have different values of conversion to microbial protein. Protozoa mostly feed upon bacteria for nitrogen source. Out of the total of amino acid pool, 54.4 - 91.8% quantity is of bacterial origin and the rest is of protozoal origin. Usually cell yield is 9 - 12 g dry matter per mole of ATP. Quantitatively ammonia level most suited for microbial utilization is 8.5 mg/100 ml. Sulphur and zinc have also been shown to stimulate microbial activity. Diets containing urea as the sole source of nitrogen do not provide adequate quantity of certain essential amino acids through microbial protein. Protection of high quality proteins from microbial attack such as casein is usually beneficial. The common methods practised are heat treatment, tannic acid treatment and formaldehyde treatment. Excess tannins may be harmful especially the condensed type which forms a complex with proteins and indigestible in the lower part of gastro-intestinal tract.

SOME STUDIES ON NUCLEIC ACIDS AND INCORPORATION
OF ^{35}S IN THE MICROBIAL PROTEIN SYNTHESIS IN
BUFFALO RUMEN

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Three adult fistulated buffaloes were taken. A replicated 3 x 3 Latin square design involving switch-over was adopted. The three treatments included 3 levels of protein viz., 80, 100 and 120 % as recommended by NRC (1971).

The rumen samples were collected at 0, 2, 4 and 6 hours for the estimations of nucleic acids (RNA-N and DNA-N), total and particulate nitrogen concentration and microbial protein synthesis in the rumen.

The RNA-N and DNA-N concentrations were minimum at 0 hour and increased to maximum at 6 hour interval. The treatment effect and interval effects were highly significant ($P < 0.01$).

The variation for the total nitrogen concentration in SRL was significantly higher ($P < 0.01$) between various treatments. High diurnal variations were observed in the total nitrogen concentration of the rumen content. Similar significant ($P < 0.01$) differences were observed in $\text{NH}_3\text{-N}$ concentrations between various treatments.

A linear relationship and a fairly constant proportionality between nucleic acids and total microbial protein (approx. 15%) was observed in the ruminal digesta and hence the total nucleic acid concentrations may be used as an index for evaluating the microbial protein synthesis in the rumen.

In the second phase fistulated Murrah buffaloes were used as a donor of the inoculum for the in vitro experiment. The animals were fed isonitrogenous urea based diets (having N:S ratio viz., 20:1, 10:1 & 5:1). For in vitro incubations three isonitrogenous urea based purified diets, varying in their sulphur levels to give three N:S ratios viz., 20:1, 10:1 & 5:1 along with ^{35}S labelled $\text{Na}_2^{35}\text{SO}_4$ were used as treatment substrates.

The percentage incorporation of ^{35}S into protein precipitate was maximum for N:S ratio 10:1, which was significantly higher ($P < 0.01$) than other treatments. Similarly, ratio of ^{35}S (CPM x 10^6)/100 mg protein precipitate N was also maximum for N:S ratio 10:1. The net increase in protein precipitate in mg/24 hour followed same trend as percentage ^{35}S incorporation and ^{35}S /protein N ratio.

CORRELATION BETWEEN DIETARY NITROGEN,
LEVEL OF PLASMA UREA AND UREA EXCRETION IN
URINE IN BUFFALO CALVES

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Two experiments were conducted with 6 male buffalo calves of Murrah breed, aged $1\frac{1}{2}$ years, to determine whether blood urea nitrogen and urea excretion in urine were associated with protein intake. The levels of urea N in the blood, excretion of ^{14}C from urea and urea N in the urine were estimated. Close relationships were observed between nitrogen intake and blood urea nitrogen and urea excretion in the urine. The observations indicate that blood urea-N and urea excretion in urine could be quantitated with the protein intake.

EVALUATION OF FODDER TREE LEAVES FOR
SHEEP AND GOAT IN SEMIARID AREA OF RAJASTHAN

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Rajasthan ranks third in India in Animal wealth. More than 60 per cent of the Livestock of the State is concentrated in the arid areas of Western Rajasthan. Sheep and goats are the major livestock and are raised on available pastures, shrub and tree leaves. Crude protein of tree leaves varies from 7 to 16.9 per cent on dry matter basis, which is almost equivalent to Berseem legume, Pala (Zizyphus numularia) and Khejari (Prosopis-cineraria) are the common fodder tree leaves available to sheep and goats. The leaves contain 5.84, 4.49 DCP and 47.51 and 40.91 TDN, respectively. These leaves failed to maintain the body weight due to poor palatability and total digestible nutrients whereas the tree leaves of Ardu (Milanthus excelsa Roxb), Siras (Abizzia lebbek) and Pipal (Ficus religiosa) have been found to maintain the body weight of the sheep due to higher available DCP and TDN.

CONCENTRATION OF VARIOUS NITROGENOUS FRACTIONS
IN THE RUMEN AS INFLUENCED BY DIETARY N:S RATIO

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Three fistulated tharparkar cows and three fistulated murrah buffaloes were fed with three isonitrogenous urea based diets varying only in their sulphur content (which was adjusted by Na_2SO_4) to give three N:S ratio of 20:1 (treatment I), 10:1 (treatment II) and 5:1 (treatment III) in a replicated 3 x 3 latin square switch over experiment. After feeding the animals on their respective rations for 4 weeks, the rumen liquor samples were drawn at 0, 2, 4, 6 and 8 hour after feeding on three alternative days and analysed for different N-fractions, TVFA and pH.

The total N concentration in SRL (strained rumen liquor) was significantly more ($P < 0.01$) with N:S ratio 10:1 and 5:1 than with 20:1. Buffalo had significantly higher ($P < 0.01$) concentration of total N (98.86 mg) than cows (91.4 mg)/100 ml SRL. The peak concentration was recorded at 2 hours after feeding. A similar pattern was observed in NH_3 -N, NPN and TCA precipitated N concentrations. The average values for NH_3 -N concentrations for the treatments I, II and III were 12.02, 14.37 and 13.44 mg for cows and 14.89, 18.01 and 17.67 mg/100 ml SRL for buffaloes, respectively.

The average NH_3 -N values for cows and buffaloes were 13.26 mg and 16.86 mg/100 ml SRL, respectively. The average TCA precipitated N figures were 51.99, 54.95 and 54.70 mg/100 ml for cows, and 55.35, 59.20 and 58.90 mg/100 ml SRL for buffaloes, for the treatment I, II and III, respectively. The concentration for TCA-N was 53.88 mg for cows and 57.74 mg for buffaloes. The TVFA concentration was also significantly higher ($P < 0.01$) in buffaloes (13.02 mg) than cows (10.57 eq)/100 ml SRL. The TVFA concentration (peak) was observed at 6 hour after feeding in all the treatments and both the species. The pH - TVFA values gave an inverse relationship. The present studies indicate that buffalo seems to be superior over cow in respect of urea utilization and N:S ratio 10:1 seems to be most desirable in the diets of cows and buffaloes.

INFLUENCE OF TANNINS ON RUMEN METABOLISM

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Experiments were conducted to investigate the influence of tannins on rumen microbial pattern of fermentation.

Four adult fistulated female buffaloes were randomly distributed in a switch over Latin Square design. The treatments I (Control) II, III and IV, respectively contained 0, 1.25, 2.5 and 5.0 % tannins made available from 0, 14, 28 and 40% salsedmeal in the rations. In treatment IV pure tannic acid was also added to get 5% total tannins. The DCP and TDN contents of the rations were kept constant. Protein and nucleic acid-N and total volatile fatty acid (TVFA) levels in the strained rumen liquor (SRL) were determined. Further, RNA-N: protein-N and total nucleic acid-N: protein-N ratios in bacteria separated from SRL were also ascertained.

The results revealed that RNA nitrogen and TVFA levels in treatment I were significantly higher than in treatment IV, whereas the protein nitrogen content in treatments II, III and IV were greater than in control group I. DNA levels were significantly lower in treatments III and IV than in treatment I. Presence of tannins in the rations resulted in an increase in protein-N but progressively depressed RNA-N and DNA-N levels with less production of TVFA.

The protein-N, RNA-N and DNA-N contents in bacteria/100 ml of SRL decreased as levels of tannins in the rations increased. The differences were significant only between treatments I and IV. But the calculated RNA-N: protein-N and total nucleic acid-N: protein-N ratios were not significantly different.

From the values obtained for nucleic acid-N and nucleic acid-N: protein-N ratios in separated bacteria, the contribution of microbial protein-N to tungstic acid precipitate of SRL, which contained both microbial and dietary protein-N, was calculated. The values obtained were: 83.87, 74.01, 63.58 and 51.14% for treatments I, II, III, IV, respectively.

The tannins in feed partially inhibited the dietary proteins from microbial attack and hence dietary protein-N content in SRL increased. Simultaneously due to the limitations imposed by tannins, microbial multiplication was adversely affected. Nevertheless, the present investigations show that salsed meal upto 28% in ration has very little harmful effect on microbial activities. This can be considered as a safe upper level for feeding purposes.



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