TISSUE DISTRIBUTION AND EXCERETION PATTERN OF AFLATOXINS AND THEIR EFFECTS ON PROTEIN METABOLISM IN RUMINATNTS

A THESIS

SUBMITTED TO THE KURUKSHETRA UNIVERSITY
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

DAIRYING

(ANIMAL NUTRITION)
IN THE FACULTY OF DAIRYING, ANIMAL HUSBANDARY
AND AGRICULTURE

BY

RAM SARAN DAS, M.Sc. (DAIRYING)

DIVISION OF DAIRY CATTLE NUTRITION
NATIONAL DAIRY RESEARCH INSTITUTE
KARNAL (HARYANA) INDIA

1988

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This Volume is Dedicated

to

the Loving and Cherishing

Memory of my Father

Shri Phagu Ram Gupta

DR. S.P. ARORA
M.S., Ph.D. (Illinois)
Additional Director of Research

Himachal Pradesh Agricultural University
Palampur 176 062 (Himachal Pradesh)

Dated, the Sept. 15, 1988

I certify that the work reported in this thesis entitled "TISSUE DISTRIBUTION AND EXCRETION PATTERN OF AFLATOXINS AND THEIR EFFECTS ON PROTEIN METABOLISM IN RUMINANTS" was carried out by Mr. RAM SARAN DAS under my guidance for the requirement of the degree of DOCTOR OF PHILOSOPHY(ANIMAL NUTRITION) in the Faculty of Dairying, Animal Husbandry and Agriculture of the Kurukshetra University, Kurukshetra.

(S.P. ARORA)

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(R.S. DAS)

C O N T E N T S

LIST OF TABLES

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LIST OF APPENDIX TABLES

LIST OF FIGURES

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LIST OF ABBREVIATIONS USED

AF/Af	:	Aflatoxin
A.M.	:	ante meridiem; before noon
AOAC	:	Association of Official Analytical Chemists
BOD	:	Biological oxygen demand
°C	:	Degree celsius
CD	:	Critical difference
cm	:	Centimeter
CP	:	Crude protein
CPM	:	Counts per minute
d	:	Day
DNA	:	Deoxy-ribonucleic acid
DM	:	Dry matter
EE	:	Ether extract
et al	:	et alii; and others
Fig.	:	Figure
FM	:	Fresh matter
g	:	Gram
h	:	Hour
Ig	:	Immunoglobulin
IU	:	International unit
i.v.	:	Intra-venous
Kg	:	Kilogram
kg $W^{0.75}$	:	Metabolic body weight
KS	:	Karan Swiss
l	:	Litre
LD_{50}	:	Mean lethal dose
m	:	Meter
M	:	Murrah
µCi	:	Microcurie
mCi	:	Millicurie
µg	:	Microgram

contd.....

mg	:	Milligram
ml	:	Millilitre
mM	:	Millimole
N	:	Nitrogen
NFE	:	Nitrogen free extract
NH_3-N	:	Ammonia-N
NRC	:	National Research Council
OM	:	Organic matter
%	:	Percent
P.M.	:	Post meridiem; afternoon
ppb	:	Parts per billion
ppm	:	Parts per million
rpm	:	Rotation per minute
SE	:	Standard error
SRL	:	Strained rumen liquor
TDN	:	Total digestible nutrients
TVFA	:	Total volatile fatty acids
U.V.	:	Ultra-violet
wt	:	Weight

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chapter 1

I N T R O D U C T I O N

1. INTRODUCTION

Aflatoxins are a group of highly toxic and carcinogenic secondary metabolites produced by certain strains of Aspergillus flavus and Aspergillus parasiticus. These fungi grow rapidly under favourable conditions of temperature and humidity on a wide variety of feeds. The toxins gained importance in 1960 when severe outbreak occurred among young turkeys within a very short space of time in England. The cause of heavy mortality among these birds resulted with the inclusion of Brazillian groundnut meal as a protein supplement in their diet. Workers at the Central Veterinary Laboratory, Weybridge, isolated the toxic factor from the toxic meal, while the scientists at Tropical Product Institute, cultured the Aspergillus flavus and from this culture produced a factor which was similar to that of toxic groundnut meal. This toxic factor was given the name of aflatoxins as a result of detailed investigations of various research workers (Loosmore and Maykson, 1961; Sargeant et al., 1961; Van Der Zijden et al., 1962; Nesbitt et al., 1962; Carnaghan et al., 1963; Allcroft and Lewis, 1963a; Barnes and Butler, 1964; Asao et al., 1965).

Aflatoxins are a group of closely related heterocyclic compounds and are potent hepatotoxins. Aflatoxin B_1 is the most preponderant of the naturally occurring

aflatoxins and is the most toxic (Wogan and Shank, 1970). Most of the animal species are affected by aflatoxins but the effects vary with dose, duration of exposure, animal species, breed and nutritional status of the animal.

Aflatoxin toxicity, known as aflatoxicosis has been reported in young turkeys (Blount, 1961); duckling and chicks (Asplin and Carnaghan, 1961); swine (Harding et al., 1963), dog (Newberne et al., 1966), calves (Loosmore and Markson, 1961), monkey (Cuthbertson et al., 1967) and trout (Edds, 1973). Aflatoxicosis displays a wide variety of symptoms in different species which includes lack of appetite (Allcroft and Carnaghan, 1963), reduced growth (Lewis et al., 1967), weight loss and general unthriftiness (Loosmore and Markson, 1961). The prolonged feeding of aflatoxin at low concentrations can lead to hepatic lesions, reduced growth rate, less production and decreased resistance to infectious diseases (Newberne, 1973).

It is now well recognised that aflatoxins display the highest carcinogenic effect in different species of animals (WHO, 1979). These toxins are not only harmful to animals but equally dangerous to human beings also because of consumption of animal originated foods such as milk, meat and egg (Masri et al., 1967; Polan et al., 1974; Stoloff, 1977; Stubblefield et al., 1983). Outbreaks of hepatitis with high rates of mortality have also been described in India (Krishnamachari et al., 1975) and Kenya (Ngindu et al., 1982) as a result of ingestion of aflatoxin contaminated diet.

Though voluminous literature has accumulated on certain aspects of aflatoxin since their discovery, yet the information is lacking otherwise, particularly with regards to the effect of dose level on metabolic aspects. Toleration limit of aflatoxins in different species especially for their young ones are unknown (Keyl and Booth, 1971). Certain dietary nutrients having protective effects against aflatoxicosis are required to be elucidated and whether there is any effect of its varying dose on nutrient utilization.

The present research studies were undertaken with the following objectives:

1. To determine the protective effect of selenium on cellulose digestion and protein synthesis in aflatoxin containing medium.

2. To find out the aflatoxin toleration limit in buffalo calves and kids.

3. To study the effect of aflatoxin on nitrogen dynamics in cattle and buffalo.

4. To investigate the tissue distribution and excretion pattern of aflatoxins in lactating goats.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Aflatoxins are a group of lactones and lactone derivatives, which are produced by several strains of Aspergillus on various feedstuffs. Investigations on aflatoxins were initiated in 1960 when 'Turkey x disease' caused heavy mortality in turkeys, pheasants, partridges and ducklings in England. Since then, efforts of various research workers, throughout the world resulted in accumulation of valuable information. An effort has, therefore, been made to review the relevant literature under the following subheads:

2.1 Chemistry of aflatoxins and their metabolites

Major aflatoxins

2.1.1 Aflatoxins B_1, B_2, G_1 and G_2 .

2.1.2 Aflatoxins M_1 and M_2 .

2.1.3 Aflatoxins GM_1 and GM_2 .

2.1.4 Aflatoxins B_{2a} and G_{2a} .

2.1.5 Aflatoxin B_3 or Parasiticol.

2.1.6 Aflatoxin R_0 or Aflatoxicol.

2.1.7 Aflatoxin P_1 .

2.1.8 Aflatoxin Q_1 .

2.1.9 Compounds D_1 and D_2 .

2.2 Structure - activity relationship

2.1 CHEMISTRY OF AFLATOXINS AND THEIR METABOLITES

Chemically, aflatoxins are highly substituted coumarins. They were named so because they were first isolated from the fungus Aspergillus flavus (A. flavus toxins). Four major aflatoxins are produced - B_1, B_2, G_1 and G_2. Several metabolites and derivatives of aflatoxins have

been isolated and characterised. Chemical structures of major aflatoxins, their metabolites and derivatives are presented in Fig. 1. A list of physical properties of aflatoxins appear in Table 1.

Major aflatoxins

2.1.1 Aflatoxins B_1, B_2, G_1 and G_2:

Nesbitt et al (1962) obtained two components from contaminated groundnut meal extract by means of chromatographic analysis, one showing a blue fluorescence and the other a green fluorescence under u.v. light, which were called aflatoxin B and G. Later on it was demonstrated that there were two blue (B_1 and B_2) and two green (G_1 and G_2) compounds produced by Aspergillus flavus (Hartley et al., 1963). Van Der Zijden et al. (1962) isolated aflatoxin B_1 in crystalline form. Asao et al. (1963, 1965) reported that the cyclopentane ring system in B_1 or the δ-lactone ring system in G_1 was arranged around the coumarin system. Carnaghan et al. (1963) observed that the reduction of isolated double bond in the terminal dahydrofuran rings of aflatoxin B_1 and G_1 resulted in aflatoxin B_2 and G_2 respectively. Presently, it is known that these four forms are naturally occurring aflatoxins on a wide variety of feedstuffs.

2.1.2 Aflatoxins M_1 and M_2:

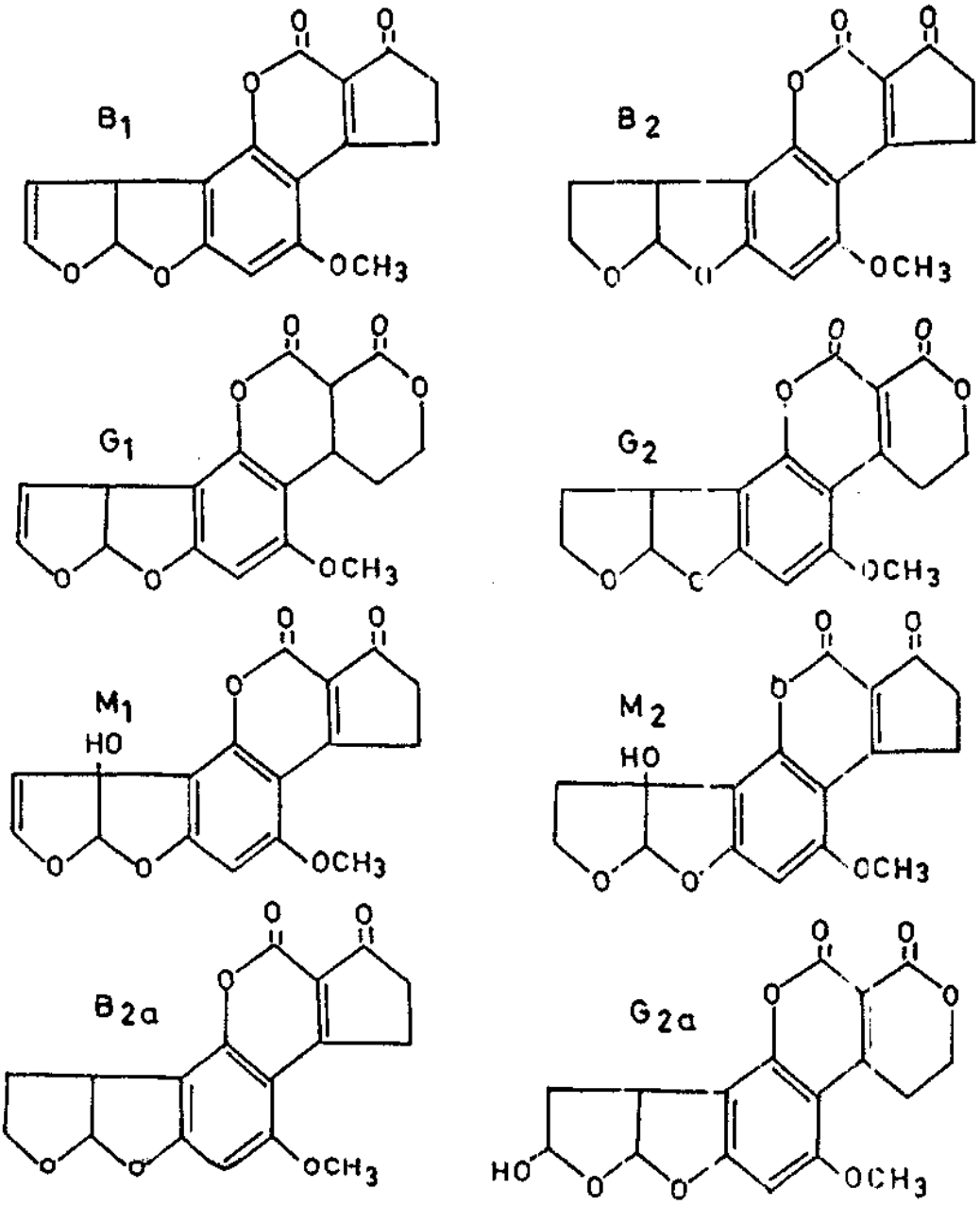
These compounds were the first hydroxy aflatoxins to be isolated and characterised as reported by Allcroft and Carnaghan (1963) and de Iongh et al. (1964) to be in

Table 1. Some physico-chemical properties of the aflatoxins*

Aflatoxin	Formulae	Mol. wt.	Crystals	M.P. (°C)	Fluoresence under UV	$^{a}R_f$ x 100
Difurocoumarocyclopentenone series:						
B_1	$C_{17}H_{12}O_6$	312	Pale yellow	267	Blue	56
B_2	$C_{17}H_{14}O_6$	314	White needles	303-306	Blue	53
B_{2a}	$C_{17}H_{14}O_7$	330	Yellow plates	240	Blue	13
M_1	$C_{17}H_{12}O_7$	328	Colourless rectangular plates	299	Blue	40
M_2	$C_{17}H_{14}O_7$	330	-do-	293	Blue	30
M_{2a}	$C_{17}H_{14}O_8$	346	Yellow rectangular plates	248	Turquoise	
Difurocoumarolactone series:						
G_1	$C_{17}H_{12}O_7$	328	Colourless needles	257-259	Turquoise	46
G_2	$C_{17}H_{14}O_7$	330	-do-	237-240	-do-	46
G_{2a}	$C_{17}H_{14}O_8$	346	Pale yellow	190	-do-	11
GM_1	$C_{17}H_{14}O_8$	344	Pale yellow plates	276	-do-	12
GM_2	$C_{17}H_{14}O_8$	346	Colourless rectangular plates	270-272	-do-	

* Heathcote and Hibert (1978); a = solid phase:silica gel G. Solvent system: chloroform-methanol (97:3, v/v)

FIG.1. STRUCTURES OF MAJOR AFLATOXINS AND THEIR METABOLITES



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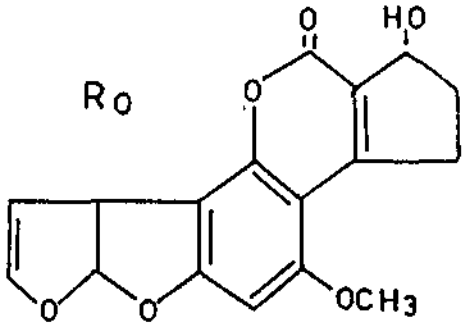
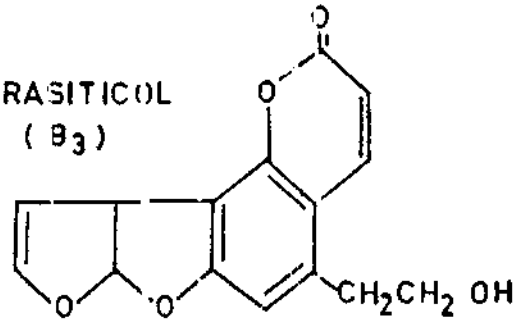
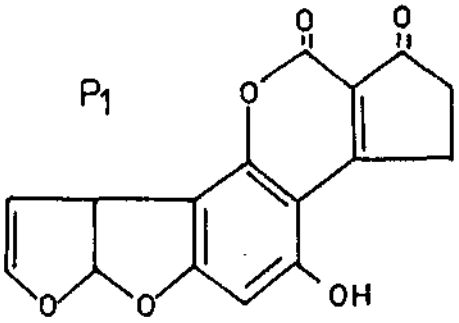
R_0



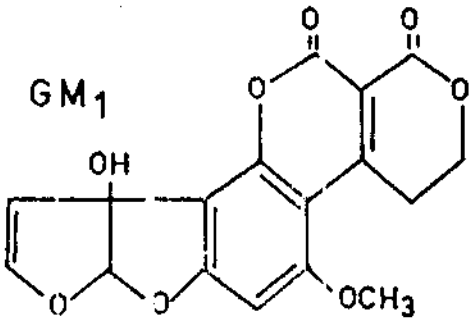
PARASITICOL
(B_3)



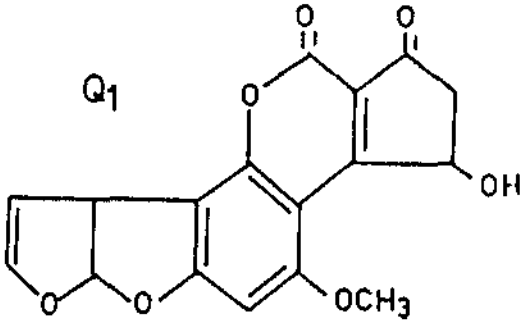
P_1



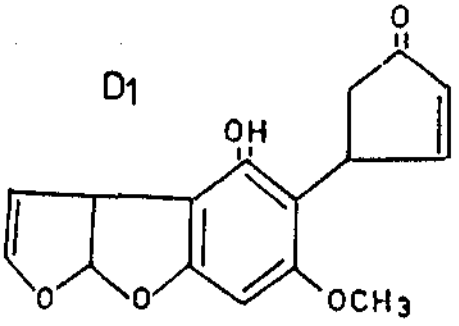
GM_1



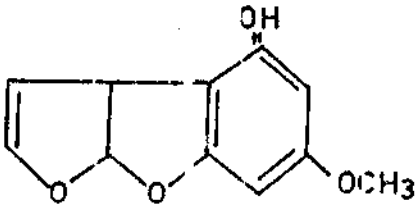
Q_1



D_1



D_2



milk of cows fed toxic peanut meal. Butler and Clifford (1965) detected a similar compound in blood and liver of rats treated with intraperitoneal injection of gastric intubation of aflatoxin B_1. It was apparent that aflatoxin was metabolised in liver to form milk toxin. Allcroft et al. (1966) observed a similar factor in the liver, kidney, milk and urine of sheep given mixed aflatoxin in their diet. Holzapfel et al. (1966) isolated the compound from sheep urine and mouldy peanuts. They further observed by micro-analysis and mass spectroscopy that M_1 has empirical formula $C_{17}H_{12}O_7$ and thus contains one oxygen atom more than aflatoxin B_1. Aflatoxin M_1 was 4-hydroxy aflatoxin B_1 and M_2 is 4-hydroxy aflatoxin B_2 (Holzapfel et al., 1966; Masri et al., 1967; Buchi and Weinreb, 1969). These hydroxylated derivatives were also isolated from lactating rat (de Iongh et al., 1964) and were as toxic as the aflatoxins from which they were derived (Purchase, 1967).

2.1.3 Aflatoxins GM_1 and GM_2:

Aflatoxin GM_1 was detected in cultures of Aspergillus flavus as a green fluorescent spot running slightly below M_1 on a thin layer chromatogram (Heathcote and Dutton, 1969; Dutton and Heathcote, 1969). A minor metabolite, aflatoxin GM_2 was also separated from aflatoxin GM_1 on further fractionation (Heathcote and Hibert, 1974).

2.1.4 Aflatoxins B_{2a} and G_{2a}:

Dutton and Heathcote (1966, 1968) observed that hydroxylation of aflatoxin B_1 and G_1 resulted in the formation of their hemiacetal derivatives, aflatoxins B_{2a} and

G_{2a} . The hydroxy-aflatoxins were somewhat unstable and tended to decompose to yellow products in the presence of air, light and alkali. Aflatoxin B_{2a} can be synthesized by chemical treatment of aflatoxin B_1 with dilute acid (Pohland et al., 1968) or by exposing aflatoxin B_1 to u.v. light in the presence of water (Wei and Chu, 1973). Pohland et al. (1968) obtained yield upto 90 per cent of aflatoxin B_{2a} by refluxing in acetone, solution of B_1 with 10 per cent sulphuric acid. Aflatoxin B_{2a} is a major metabolite of aflatoxin B_1 in several species (Patterson and Allcroft, 1970; Patterson and Robert, 1970).

2.1.5 Aflatoxin B_3 or Parasiticol:

Aflatoxin B_3 or the compound called 'Parasiticol' was isolated independently by three different groups of workers (Heathcote and Dutton, 1969; Stubblefield et al., 1970; Cole and Kriksey, 1971). It was a natural metabolite of Aspergillus flavus (Heathcote and Dutton, 1969) and Aspergillus parasiticus (Stubblefield et al., 1970) and it arose as a product of the metabolism of aflatoxin G_1 by Rhizopus species of mould (Cole and Kriksey, 1971).

2.1.6 Aflatoxin B_0 or aflatoxicol:

Aflatoxicol was isolated as a degradation product of aflatoxin B_1 by certain micro-organisms (Detroy and Hesseltine, 1970; Robertson et al., 1970). The aflatoxin metabolites, aflatoxicol and dihydroaflatoxicol are formed by the reduction of the carbonyl group of the terminal cyclopentane of aflatoxin B_1 and B_2 respectively.

Aflatoxicol is also formed by the reduction of aflatoxin B_1 by lithium tris aluminium hydride (Pawlowski et al., 1977). Aflatoxicol consists of two metabolites, aflatoxicols A and B, which have been shown to produce many of the toxic effects of B_1. Aflatoxicol A is more toxic than aflatoxicol B.

2.1.7 Aflatoxin P_1:

Dalezios et al. (1971) identified a new phenolic derivative of aflatoxin B_1 in a conjugated form, designated as aflatoxin P_1. This metabolite is formed by the process of O-demethylation of the parent compound. Aflatoxin B_1 is excreted mainly as aflatoxin P_1 in Rhesus monkey (Dalezious et al., 1971) and to a significant extent in rats (Wogan et al., 1967).

2.1.8 Aflatoxin Q_1:

Masri et al. (1974) identified another hydroxylated derivative of aflatoxin B_1, when aflatoxin B_1 was incubated with monkey liver microsomal preparations in phosphate buffer. This new metabolite was named as aflatoxin Q_1. Aflatoxin Q_1 possesses a hydroxyl group which is in the β-position to the carboxyl group of cyclopentane ring in aflatoxin B_1. It contains one oxygen atom more than B_1 (Steyn et al., 1974).

2.1.9 Compounds D_1 and D_2:

Lee et al. (1974) isolated in crystalline form the major product formed during the reaction of pure aflatoxin

B_1 with ammonium hydroxide at 100°C under pressure. The compound having a molecular weight of 286 was non-fluorescent and lacked the lactone group characteristics of aflatoxin B_1 . It was postulated that the new product $C_{16}H_{11}O_5$ arose from opening of the lactone ring of aflatoxin B_1 during ammoniation. Since this compound was derived from decarboxylation, the trivial name aflatoxin D_1 was proposed for this metabolite.

Cucullu et al. (1976) isolated and characterised a second compound formed during ammoniation reaction. This product called as D_2 was also non-fluorescent phenol of low molecular weight (206) than D_1 but lacked the lactone carbonyl and cyclopentanone ring, characteristic of aflatoxin B_1 . They further suggested that the most likely way in which these compounds formed were by the opening of the lactone ring of aflatoxin B_1 , followed by decarboxylation of the resultant β-keto acid to produce both compounds D_1 and D_2 . These compounds were suggested to be called as derived aflatoxins because of their formation during the ammonical detoxification of aflatoxin B_1 (Heathcote and Hibert, 1978).

2.2 STRUCTURE-ACTIVITY RELATIONSHIP

The various aflatoxins and their derivatives described above differ in their relative toxicity. A comparative data on their activity is useful in identifying the structural features of the molecule which controls the aflatoxin potency. There is considerable body of data on the relative activities of major aflatoxins in evoking specific biological

and biochemical responses (Ayres et al., 1971; Wogan et al., 1971; Wong and Hsieh, 1976). In large majority of cases a clear-cut potency of the series - $B_1 > G_1 > B_2 > G_2$ is evident.

The above mentioned information indicates that certain structural features are responsible for the activity of aflatoxins in biological systems. Clifford and Rees (1966) observed that the furofuran ring attached to the coumarin nucleus is essential for toxic and carcinogenic responses. Compounds lacking the furofuran portion were inactive in every system (Lin et al., 1977).

The compounds containing lactone ring attached to the coumarin nucleus (e.g. aflatoxins of G series) are less potent than the aflatoxins of B series which contain instead a dihydrofuran moiety. The presence of a double bond between 2 and 3 positions in the dihydrofurofuran segment of the molecule is important. The reduction of this double bond (as in aflatoxins B_1 to B_2 and G_1 to G_2) brings about significant reduction in the potency. Detroy and Hesseltine (1968) reported that the reduction of ketone moiety of the terminal cyclopentanone ring of aflatoxin B_1 to form aflatoxicol caused loss in potency. McCann et al. (1975) found that 4-hydroxylation of aflatoxin B_1 to form aflatoxin M_1 greatly reduced its mutagenecity and carcinogenicity (Canton et al., 1975). Nearly complete abolition of toxicity and mutagenecity has been reported with hydroxylation of aflatoxin molecule at other positions as in aflatoxins P_1 and Q_1 and aflatoxicol H_1 (Hsieh et

al., 1974; Salhab and Hsieh, 1975; Stoloff et al., 1972). Relatively high mutagenic potency of the hydroxylated metabolite, aflatoxicol could be attributed to its metabolic conversion to aflatoxin B_1 (Patterson and Roberts, 1972; Salhab and Edwards, 1977a).

2.3 BIOSYNTHESIS OF AFLATOXINS

Adye and Mateles (1964) studied the biosynthesis of aflatoxin and observed the incorporation of ^{14}C labelled methionine, phenylalanine, tyrosine, tryptophan and acetate into aflatoxin B_1. Studies on biosynthesis of aflatoxin B_1, the major metabolite of most aflatoxigenic molds have shown that the basic skeleton of the toxin molecule is derived entirely from acetate units via the polyketide pathway and that methionine contributes the methoxy-methyl group (Biollaz et al., 1970; Hsieh and Mateles, 1970).

Holker and Underwood (1964) report that aflatoxins are derived from sterigmatocystein which is a toxic secondary metabolite of Aspergillus versicolor. Holker and Kagal (1968) suggested that sterigmatocystein was biologically derived from versicolorin A or a related compound. But Tanabe et al. (1970) showed that sterigmatocystein was biogenetically derived from two separate polyketide units. Hsieh et al. (1973) found that labelled sterigmatocystein was converted to aflatoxin B_1 by the resting mycellium of Aspergillus parasiticus.

Lin and Hsieh (1973) and Lin et al. (1973) converted labelled averufin into aflatoxins B_1, B_2, G_1 and G_2.

The conversion of versicolorin A and norsolorinic acid into aflatoxin has been observed (Hsieh et al., 1976; Lee et al., 1976). Thus, norsolorinic acid, averufin, versicolorin A and sterigmatocystein have been shown to be the biogenetic precursor of aflatoxins.

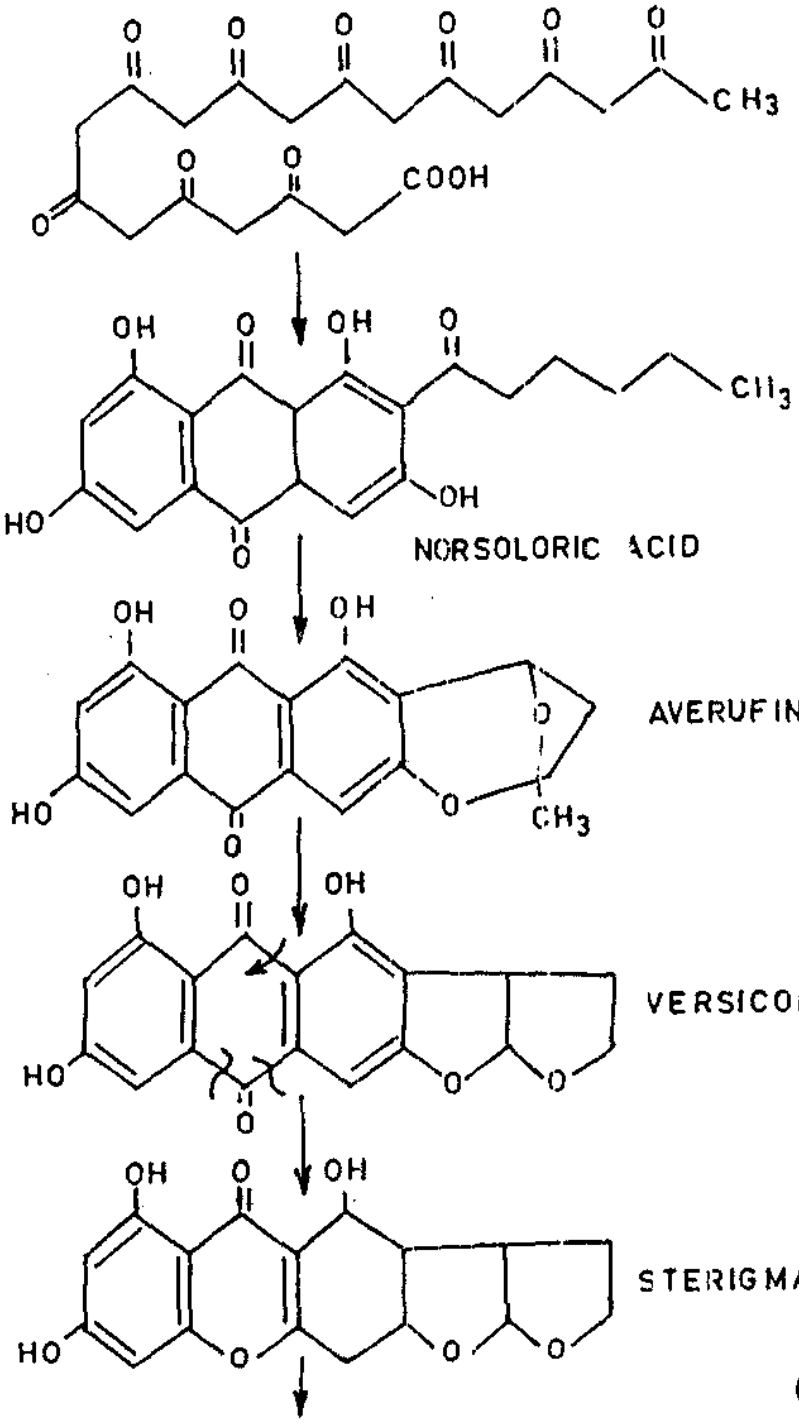
Biollaz et al. (1968,1970) suggested a common pathway for the synthesis of sterigmatocystein and aflatoxin B_1. They later postulated a biosynthetic route for aflatoxin B_1 from acetate-malonate derived polyhydroxy napthacene endoperoxide. A schematic pathway for aflatoxin biosynthesis is given in Fig. 2.

2.4 FACTORS AFFECTING AFLATOXIN PRODUCTION

Spores of Aspergillus flavus are found in high proportions in many agricultural commodities which are used as animal feeds. However, cultures of Aspergillus flavus grown on oilseeds seem to have a greater potential for aflatoxin production than those isolated from cereal products. This is confirmed in field surveys that the highest levels and frequency of aflatoxin contamination occur in groundnuts and cottonseeds. However, substrate is only one factor which determines the synthesis of aflatoxins. The other important factor is the relative humidity and environmental temperature.

Austwick and Ayerst (1963) observed that high relative humidity was the most important factor for the growth of Aspergillus flavus on a natural substrate. Diener and Davis (1969) reported that a few fungi grew at a humidity

FIG.2. A SCHEMATIC BIOSYNTHETIC PATHWAY OF AFLATOXINS



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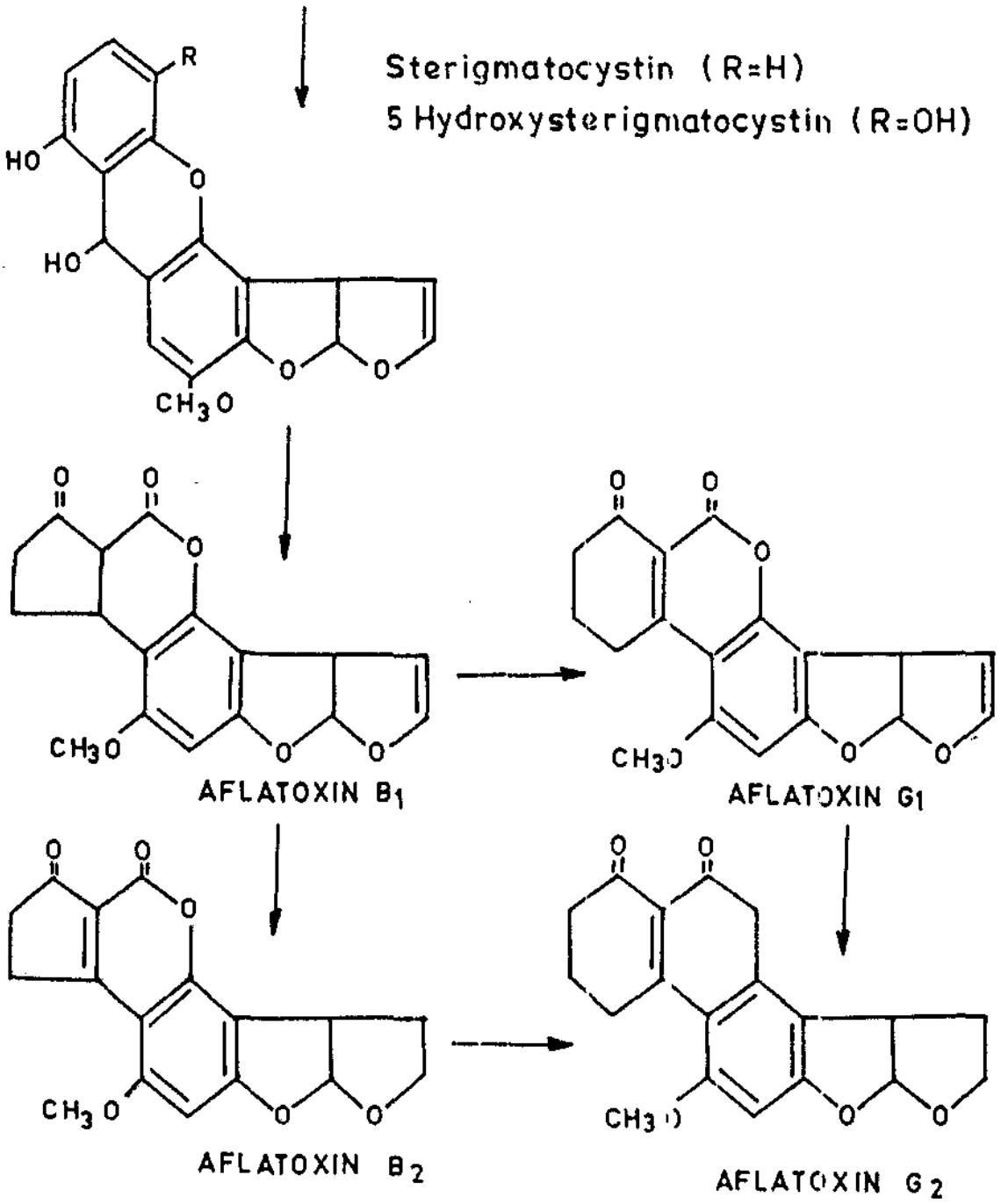

Sterigmatocystin (R=H)
5 Hydroxysterigmatocystin (R=OH)
R
HO
HO
O
O
O
CH_3O
AFLATOXIN B_1
AFLATOXIN G_1
AFLATOXIN B_2
AFLATOXIN G_2

less than 70 per cent. At 85 per cent humidity and at a moisture level of 30 per cent in groundnut, the aflatoxin content increased tremendously. When the moisture level was reduced to 15 per cent after drying and at a relative humidity of 50 per cent, little aflatoxin was produced.

Rabie and Smalley (1965) reported optimum temperature of 24°C for aflatoxin B_1 and 30°C for G_1. Sorenson et al. (1967) observed that when aflatoxin B_1 and G_1 were produced on rice, an increasing temperature resulted in more toxin production. The production of toxin in the stored corn was enhanced when temperature was above 25°C and moisture level above 16 per cent (Shotwell, 1977).

On lipid commodities, the minimum conditions for aflatoxin production are a moisture content of 9-10 per cent whereas for the starchy cereal grains the lower limit for aflatoxin production is 13.3-13.5 per cent (Davis and Diener, 1970).

2.5 RELATIVE FORMATION OF AFLATOXINS B_1, B_2 G_1 & G_2

Different strains of the Aspergillus flavus subgroup show considerable variations in the type and amount of aflatoxin production. The amount and relative proportions of the four major aflatoxins B_1, B_2, G_1 and G_2 depends upon the strain, balance of nutrients, culture conditions and isolation procedure used (Applegate and Chipley, 1974; Hesseltine et al., 1966, 1970; Reddy et al., 1972; Mehan and Chohan, 1973).

Strains producing only the B and G groups of

aflatoxins are well documented (Diener and Davis, 1966; Diener and Davis, 1969; Maggon et al., 1969). Papa (1977) found some strains of Aspergillus flavus which produced only aflatoxin B_2. However, strains capable of producing only G_1 or G_2 have not been reported so far in the literature. This seems to suggest that aflatoxin G_1 is derived from aflatoxin B_1 by further oxidation (Biollaz et al., 1970).

Lower pH and trace metals like manganese and vanadium were found to favour formation of hydroxylated aflatoxins (Maggon, 1972).

Conditions like aeration, agitation and higher temperature results in the formation of more amount of G_1 than B_1. Gupta (1973) reported a relatively large amount of aflatoxin G formation in stationary cultures as compared to shake cultures.

2.6 OCCURRENCE OF AFLATOXINS IN FEEDSTUFFS

The ubiquitous fungi Aspergillus flavus often invades and produces aflatoxin on a wide variety of feeds such as cotton seed, soyabean, corn, rice, wheat, millet, barley, sesame, sorghum, peanut, peas, beans, cowpeas, cassava and sweet potatoes.

Bean et al. (1972) observed in a survey in Maryland that 14 out of 28 samples of soyabean meed contained aflatoxin. Although maize is susceptible to contamination with aflatoxin, the levels of aflatoxin which have been reported to be present in contaminated samples are low

in comparison with those found in groundnut meal (Shotwell et al., 1973). Individual lots of groundnut meal have been found to contain levels of aflatoxin varying between 1 and 25 ppm in surveys carried out in various parts of the world (FAO, 1979).

In surveys carried out in USA and Finland (Stoloff, 1976; Krogh et al., 1970), 88 per cent of the samples tested contained aflatoxins in amounts ranging from traces to around 30 parts per billion but individual lots have been recorded as containing upto 0.5 parts per million of the aflatoxins.

Connole et al. (1981) conducted a survey on mycotoxins in animal feed in Queensland between 1971 and 1980 and found 67 samples to be contaminated with aflatoxin out of 355 samples. A survey was carried out in Pakistan by Shaw et al. (1981) and observed that 8 samples of feed ingredients out of 52 samples were positive for aflatoxin.

Sanchis et al. (1986) screened 331 samples comprising cereal grains, mixed feeds, edible nuts for the presence of aflatoxin in Spain. Aflatoxins were detected in 4 of 27 samples of mixed feeds. Maize samples were having aflatoxin invariably and showed the highest proportion of aflatoxigenic moulds.

Various surveys conducted from time to time on mycotoxins in feeds from India revealed a high level of aflatoxins in their feeds (Sreeniwasmurthy et al., 1965; Suryanarayan Rao et al., 1965; Tulpule, 1969; Yadagiri and Tulpule, 1974; Krishnamachari et al., 1975).

Pulsounder and Shukla (1977) conducted a survey in Gujarat state and found about 42 per cent of feed samples to be contaminated with aflatoxin. They observed aflatoxin in each class of feed like oil meals, cereal grains, cereal byproducts and the compound cattle feeds. Aflatoxin B_1 was present in nearly all the positive samples while B_2 and G_1 in some but aflatoxin G_2 was only in soyabean meal.

Mishra and Singh (1978) surveyed food grains in some villages of Mathura district of U.P., affected by floods in 1976. They observed that out of 36 feed samples, 18 were positive for aflatoxin. Ram Pal et al. (1979) estimated aflatoxins in groundnut oil, groundnut cake and hydrogenated oil sold in the market of Hapur U.P.). They could not detect any aflatoxin in refined groundnut oil and hydrogenated fat but 69.7 per cent of crude oil samples and 70 per cent of groundnut cake samples were positive for aflatoxin.

Patel et al. (1981) collected various oil cakes and feeds from different states of India. On analysis, it was found that about 64 and 80 per cent samples of groundnut cake and cottonseed cake were contaminated with aflatoxins. More than 50 per cent samples of maize, sorghum, rice and wheat contained aflatoxin. Aflatoxins were also detected in some samples of linseed, mahua, safflower, sesame and sunflower cakes.

Reddy et al. (1984) conducted a survey on aflatoxin contamination of some poultry feed ingredients in Andhra Pradesh. They observed that groundnut cake in general

contained higher levels (0.587 ppm) of total aflatoxin than other feeds.

Singh et al.(1984) collected 127 samples of different cattle and poultry feeds from different parts of India for aflatoxin analysis. Groundnut cake samples had the highest level of aflatoxin B_1 followed by cottonseed and compound feeds. In addition,they also observed the presence of aflatoxin B_1 in linseed cake, Bengal gram, coconut cake, wheat bran and mustard cake. Horse gram and rice bran were free from aflatoxin B_1.

Balasubramanian (1985) analysed 101 samples of animal feeds and feed ingredients collected from around Madras city and also from different parts of the Tamil Nadu state for aflatoxin. Sixty-three per cent of the samples had aflatoxin B_1 . Among the feed ingredients analysed only groundnut cake was contaminated with aflatoxin B .

Sinha and Arora (1985) analysed 68 feed ingredients and compound feeds collected from Agricultural Universities, National Institutes and Government farms in India, for the presence of aflatoxin. Groundnut cake samples showed the highest incidence of presence of aflatoxins. These samples contained aflatoxin B_1 in the range of 0.12 to 1.0 ppm and aflatoxin G_1 in the range of 0.08 to 0.10 ppm. Out of 4 samples each of cattle feed poultry mash and pig mash, 3 in each were positive for aflatoxin B_1 .

Namaiah et al. (1986) observed the presence of aflatoxin ranging from 0-660 µg/kg in wheat flour samples collected from around Bangalore.

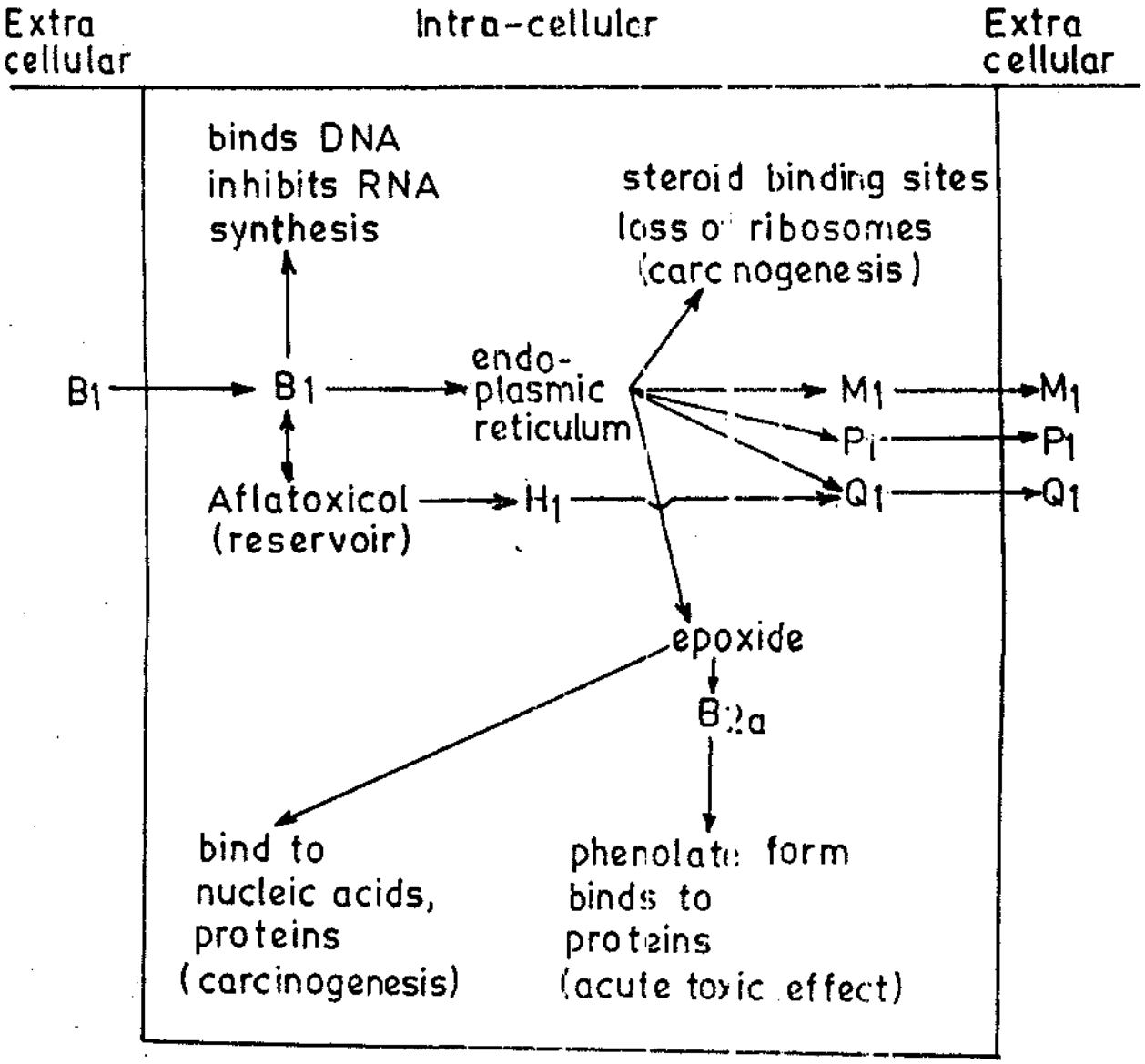
Johri et al. (1986) screened 208 samples comprising yellow maize, groundnut cake, fish meal, rice bran and complete diets for poultry, collected in and around Bareilly (U.P.) for the presence of aflatoxins. The incidence of aflatoxin B_1 was highest in groundnut cake (71.4 per cent), followed by maize (47 per cent), fish meal (25 per cent) and rice bran (16.7 per cent) samples. Starter feed mixture contained aflatoxin B_1 at 0 to 2 µg/g and the grower mixture contained upto 1.0 µg/g. Aflatoxin content of the feeds was high during the hot-humid period.

2.7 METABOLISM OF AFLATOXIN AND THEIR MODE OF ACTION

The metabolism of aflatoxins plays an important role in determining their toxicity (Campbell and Hayes, 1976). Studies on the metabolism of aflatoxins are mostly focussed on aflatoxin B_1, as it is the most abundant and most toxic member of the aflatoxins. Swenson et al. (1977) reported that aflatoxin B_1 requires metabolic activation to exert its reactivity. Campbell and Hayes (1976) report that aflatoxin B_1 may be oxidised by liver microsomal oxygenases to aflatoxin M_1, aflatoxin Q_1, aflatoxin P_1 and aflatoxin B_{2a} or may reduce by cytoplasmic reductase to aflatoxicol (Salhab and Edwards, 1977b) and aflatoxicol H_1 (Salhab and Hsieh, 1975). A generalised scheme for the metabolism of aflatoxin B_1 in the liver is given in Fig. 3 and various possible biotransformations of aflatoxin B_1 and their reactions are described below.

Aflatoxin B_1 may be metabolically activated by the

FIG. 3. GENERALISED SCHEME FOR THE METABOLISM OF AFLATOXIN B_1 IN LIVER



mixed function oxidase (MFO) to form 2,3 oxide or epoxide. This epoxide binds covalently to DNA forming ^{7}N-guanyl adduct or is hydrolysed by epoxide hydrase to the dihydrodiol. The later metabolite, in its dialdehydride phenolate form at physiological pH, forms schiff's base with proteins. The other metabolites are hydroxylated derivatives. Aflatoxin M_1, aflatoxin P_1 and aflatoxin Q_1 are produced by mixed function oxidases but aflatoxicol is formed reversibly by NADP dependent cytoplasmic dehydrogenase.

2.8 TOXICITY IN DIFFERENT SPECIES

The toxic effect of aflatoxins varies from species to species, and further depends upon age, sex, level of ingestion, feeding duration and nutritional status of the animal.

The animal species have been divided into three groups based on the lethal levels of aflatoxin B_1 as: (1) extremely susceptible - duckling, rabbit, cat, pig and trout; (2) moderately susceptible - dog, sheep and guinea pig; (3) resistant - monkey, chicken, rat, mouse and hamster. Salhab and Edwards (1977a) classified the man in the relatively resistant category because it has got similar susceptibility to aflatoxin as monkey.

Wogan and Pong (1970) reported that aflatoxin B_1 was most lethal to various species of animals followed by G_1, B_2 and G_2 in order of decreasing potency. LD_{50} values of aflatoxins and the dietary concentrations of aflatoxin causing toxicosis in animals are presented in Tables 2 and 3 respectively.

Table 2. LD_{50} values of aflatoxins in various animals (mg per kg body weight)

Species	B_1	B_2	G_1	G_2
Duckling	0.36	1.68	0.78	1.42
Rabbit	0.30			
Cat	0.55			
Pig	0.62			
Trout*	0.81		1.90	
Dog	0.50-1.00			
Sheep	1.00-2.00			
Guinea pig	1.40			
Monkey	2.20-7.80			
Chicken	6.30			
Rat	7.20 (M) 16.00 (F)			
Mouse	9.00			
Hamster	10.20			
Chicken embryo	0.025 per embryo		.00 per embryo	

* Intraperitoneal route
(others by oral route)

Table 3. Dietary aflatoxin levels causing toxicosis in farm animals[a]

Animal	Age	Aflatoxin content (ppm)	Duration of feeding	Effects
Calf	Weanling	0.22-2.2	16 weeks	Stunting, liver damage, death
Steer	2 years	0.22-0.66	20 weeks	Liver damage
Cow	2 years	2.4	7 months	Liver damage
Pig	New born	0.23	4 days	Stunting
Pig	2 weeks	0.17	23 days	Anorexia, stunting, jaundice
Pig	4-6 weeks	0.4-0.7	3-6 months	Stunting, liver damage
Chicken	1 week	0.84	10 weeks	Stunting, liver damage
Duck	Unknown	0.30	6 weeks	Liver damage, death

a Wogan (1977)

2.8.1 Cattle

Loosmore and Markson (1961) reported initial cases of poisoning in calves fed Brazillian groundnut meal. The symptoms observed were aimless walking in circles, frequent falling, ear twitching and grinding of teeth. The symptoms were worse in calves than in adult cattle. Similar symptoms were reported in cattle by Clegg and Bryson (1962).

Allcroft and Lewis (1963b) studied the effect of feeding rations containing 20 per cent of a toxic groundnut meal to calves, heifers and older cows. First abnormality in the calves was a reduction in growth rate followed by unthriftiness and loss of appetite. Terminal symptoms occurred after 16 to 25 weeks. In heifers, loss of condition occurred after 7 months, while no clinical abnormality was seen in older cows.

Horrocks et al. (1965) included upto 8 per cent of the toxic groundnut meal in concentrate diets for early weaned calves and found a significant depression in live weight gain prior to 3 months, which was due to lowered feed intake and impaired feed utilization, but could not find any adverse effect on the performance of fattening bulls fed a concentrate mixture having toxic groundnut meal.

Sastri et al. (1965) were the first to observe the incidence of groundnut toxicity in livestock in India. Their pathological findings of the animals who died due to an outbreak in Andhra Pradesh (India) were similar to

the conditions reported by Loosmore and Markson (1961). Later on, a case of acute aflatoxicosis in cattle was reported by Gopal et al. (1968).

Garrett et al. (1968) conducted an experiment on young beef cattle, divided into five groups fed graded levels of aflatoxin fortified cottonseed meal for 133 to 196 days. The levels of aflatoxin in five different groups were 0, 100, 300, 700 and 1,000 ppb respectively. Decreased growth rate alongwith increase in liver and kidney weights were recorded in animals given 700 and 1,000 ppb levels of aflatoxins.

Lynch et al. (1968) conducted an experiment to see the effect of graded levels of aflatoxin on carotene and vitamin A concentration in blood serum of calves. The calves were dosed orally with aflatoxin at rates of 0.04 to 0.10 mg/kg body weight. At aflatoxin dose levels of 0.08 mg/kg and above, carotene values showed a progressive decline during the dosing period but the vitamin A values decreased most rapidly from the first to the second week of dosing.

Flatla et al. (1969) studied the effect of feeding a concentrate mixture containing 25 to 30 per cent groundnut meal in two groups of bulls from 3 weeks of age upto 13 months. Groundnut meal containing 20 to 50 ppb of aflatoxin B_1 was used in group I. Groundnut meal containing 1,500 to 1,900 ppb aflatoxin B_1 was used in group II. The animals fed higher levels of aflatoxin did not show any clinical sign, though after 3-4 months they had less average daily gains.

Lynch et al. (1970) reported that liver lesions occurred at 0.02 mg of aflatoxin B_1 per kg body weight and above in dairy calves on a 6 weeks toxin feeding. Gross lesions at postmortem included loss of colour in the liver and adrenal hyperplasia. Aflatoxins at a level of 300 ppb in the diet of beef steers fed for 4-5 months was quite safe (Keyl and Booth, 1971).

Lynch et al. (1972) studied the lethality of a single dose of aflatoxin in calves. The dose levels ranged from 0.6 to 1.8 mg per kg body weight. All the calves survived at dose levels upto 1.0 mg. Two of the three calves survived at dose levels of 1.2 to 1.6 mg. All the calves died at the dose level of 1.8 mg.

Neathery et al. (1980) fed young dairy calves diets containing 40 to 650 ppm zinc, with or without 5 ppm aflatoxin for 3 weeks. The aflatoxin fed calves exhibited signs of toxicity which included reduced feed intake and less body weight gain. Addition of 600 ppm zinc to diets did not alleviate the toxic effects.

Eddy et al. (1980a) fed Holstein heifers a diet containing contaminated corn, providing 4.8 mg aflatoxin daily for 140 days. They found no adverse effect on feed intake, body weight gain and feed efficiency. In another trial on heifers, Eddy et al. (1980b) observed that aflatoxin dose upto 4.83 mg neither affected the metabolic profile, which included BUN, total protein, SGOT, LDH and 14 other constituents nor the length of the oestrus cycle.

Balaraman and Arora (1986) studied the effect of

feeding graded levels of aflatoxins in 4 groups of crossbred calves for 16 weeks. The toxin levels were 0, 0.5, 1.0 and 1.5 ppm respectively on the basis of dry matter intake. The results indicated a decline in weight gain with increase in aflatoxin levels. They elucidated that 0.26 ppm aflatoxin was safe for crossbred calves.

2.8.2 Sheep and goats:

Aflatoxicosis has not been reported in sheep under field conditions. Sheep appear to be relatively resistant to aflatoxin with LD_{50} value of 2 mg/kg body weight (Patterson, 1973).

Armbrecht et al. (1970) observed loss of appetite and diarrhoea in wethers, given a single sublethal dose at the rate of 0.23 mg/kg body weight and more. Excessive salivation, frothing from the mouth and increased rectal temperature within 12 h were seen at a higher dose of 1.28 mg/kg but not at 0.59 mg/kg dose.

Allcroft et al. (1966) observed the presence of aflatoxin metabolites in various tissues of sheep fed aflatoxin. They also detected the excretion of B_1, G_1 and M_1 through urine. Aflatoxin M_1 was excreted mainly through urine while aflatoxin G_1 was excreted through urine and faeces both (Nabney et al., 1967).

Lewis et al. (1967) observed in a long term feeding experiment on sheep that weight gain was slightly less and fertility was also lower due to feeding of toxic groundnut meal containing 1 to 1.75 ppm aflatoxin.

Maryamma and Sivadas (1975) reported that ingestion of feed containing 0.7 ppm of aflatoxin produced deleterious effects in adult goats. They also observed that male is more susceptible to aflatoxin than female. Intake of aflatoxin at very low levels, insufficient to produce toxic symptoms, causes testicular degeneration. Histopathological changes were also noticed in the pituitary, thyroid and adrenal glands.

Samarajeewa et al. (1975) reported the incidence of aflatoxicosis in goats fed a concentrate mixture containing Polkudde meal. They also recovered aflatoxins from the liver and urine of the affected animals.

Hatch et al. (1979) were able to induce aflatoxicosis in goats and the symptoms observed were anorexia, CNS depression, swaying and falling, shock, coma and death with or without convulsions before death.

Sinha and Arora (1984b) studied the effect of feeding graded levels of aflatoxins ranging from 0 to 1.0 ppm in the diet of kids. They observed that 1 ppm aflatoxin in the diet adversely affected the growth rate, gluconeogenesis and muscle protein synthesis. They also observed histological changes in liver, kidney and testes of the kids. In another study, Arora et al. (1988b) observed a significant decrease in blood serum immunoglobulin and growth rate in kids given 0.5 and 1.0 ppm aflatoxin in the diet. They also reported that 0.147 ppm aflatoxin in the diet of preruminant kids might be a safe level.

2.8.3 Swine:

Loosmore and Harding (1961) reported the field outbreaks in pigs due to the intake of contaminated groundnut meal. Annau et al. (1964) observed that diets containing 0.5 to 0.75 ppm aflatoxin were unpalatable to growing pigs resulting in a marked depression in growth and feed conversion efficiency.

Bodnar et al. (1965) conducted feeding trials in Hungary on pigs and found that dietary levels of 0.4 ppm or more caused a reduction in weight gain and feed conversion efficiency. Dietary levels of 0.45 ppm aflatoxin B_1 or below had no significant effect on either growth rate or feed conversion efficiency of growing pigs over a period of 117 days (Hintz et al., 1967).

The LD_{50} value of aflatoxin for swine was reported to be 0.6 mg/kg body weight by Jones and Jones (1969), while Ciegler (1975) found it to be 0.62 mg/kg body weight.

Barber et al. (1968) studied the effect of feeding aflatoxin contaminated groundnut meal on the performance of pigs. The symptoms observed were loss of appetite and reduced growth rate.

Keyl and Booth (1971) did not find any adverse effect in growing swine as a result of feeding a diet having aflatoxin level upto 233 ppb or less. Growth rate of pigs receiving aflatoxin upto 1.1 mg per kg feed was not affected (Armbrecht et al., 1971).

During the survey of aflatoxicosis in farm animals,

Smith et al. (1976) found that among all the cases of aflatoxicosis, 88 per cent were in swine.

Southern and Clawson (1979) reported that average daily gain was reduced linearly in pigs receiving a diet containing 355 ppb and higher levels. Feed efficiency was not significantly influenced by dietary levels of 385 and 750 ppb aflatoxin but was depressed at a higher level of 1,480 ppb in the diet.

Hale and Wilson (1979) noted that pigs fed aflatoxin free maize showed higher digestibility coefficients of DM, EE and NFE and more N balance than pigs fed diets with aflatoxin contaminated maize.

Panangala et al. (1986) reported that growth rate and feed conversion efficiency was significantly less in pigs fed aflatoxin as compared to control.

2.8.4 Poultry:

Among the poultry birds, chickens are relatively less susceptible than ducklings and turkey poults. Asplin and Carnaghan (1961) reported first evidence of susceptibility of duckling to the toxic principles of groundnut meal. The first signs of aflatoxicosis in duckling were inappetance and poor growth rate, with the onset of mortality 2 weeks after the commencement of the feeding of toxic groundnut meal. They also noticed retardation of growth in Rhode island chickens at dose levels of 0.5 ppm aflatoxin.

Ducklings are extremely sensitive to aflatoxins. Purchase (1967) reported that the oral 7 days LD_{50} was

18.2 µg aflatoxin B_1 and 16 µg aflatoxin M_1 in the day old duckling, whereas an acute LD_{50} for aflatoxin B_1 and G_1 was 0.335 mg and 0.784 mg/kg body weight respectively. The extreme sensitivity of ducklings provided the earliest bioassay for aflatoxin contamination of feeds and detection of aflatoxin as low as 50 µg/kg feed was possible.

Carnaghan (1965) fed ducklings a diet containing approximately 0.3 ppm aflatoxin from 7 days of age over a 14 months period. Deaths and significant depression in weight gain occurred during the first 4-5 weeks. The survived birds had hepatic tumour.

Gopal et al. (1969) recorded several cases of aflatoxicosis in chicken. The disease was more prevalent in young stock, which showed the symptoms of acute toxicity.

Smith and Hamilton (1970) reported high mortality and symptoms of aflatoxicosis within 48 hours of introduction of the mouldy maize in the chicken ration. Hamilton (1971) observed spontaneous outbreak in North Carolina, in which 50 per cent of a flock of laying hens died within 48 hours of being fed highly toxic maize containing 100 ppm aflatoxin. Hamilton et al. (1972) recorded a reduced weight gain and increased relative weights of spleen and pancreas in turkeys as a result of aflatoxin feeding at 0.125 ppm level in the diet.

Bryden et al. (1979) reported that aflatoxin B_1 (1 mg/kg diet) had a detrimental effect on body weight gain, feed intake and feed conversion efficiency in chickens. When the toxin was fed for more than 2 weeks that led to

increased relative liver weight and liver lipid concentration.

Sinha and Arora (1987) found 1 ppm level in the diet of Hyline chicks to be safe from toleration point of view, as it did not affect the growth. In another study, Arora et al. (1988a) did not find any adverse effect on chicks fed a diet supplemented with 1.71 ppm aflatoxin.

2.8.5 Humans:

Systematic studies on the effects of aflatoxins on human beings have not been carried out. However, numerous cases showing symptoms similar to aflatoxicosis have been recorded in human beings throughout the world.

Ling et al. (1967) reported death of 3 children aged 4-6 years due to the intake of mouldy rice for upto 3 weeks.

Serck-Hansen (1970) suspected the death of a 15-year old African boy in Uganda due to the intake of aflatoxin contaminated cassava. The boy had abdominal pain and swelling and died after 6 days. A histopathological examination indicated interstitial oedema of the heart, congestion and oedema of the lungs and centrilobular necrosis of liver.

Robinson (1967) analysed the urine samples of cirrhotic children in India and observed the involvement of aflatoxins in these cases. Shank et al. (1971) detected traces of aflatoxin B_1, taking specimens on autopsy from 11 of 15 control subjects, probably reflecting chronic low level ingestion of the toxin by the general population of north

eastern Thailand, an area where aflatoxin contamination of the foods was high.

Becroft and Webster (1972) observed aflatoxins B_1 and G_1 in liver extracts from two New Zealand cases who died of Rey's syndrome. They suggested that contamination of food by aflatoxin might have a role in the aetiology of Rey's syndrome.

The most convincing account of acute aflatoxicosis in man was reported from India (Krishnamachari et al., 1975). The cause of aflatoxicosis was the consumption of maize contaminated with upto 15 mg aflatoxin B_1 per kg. The persons suffering from aflatoxicosis had acute hepatitis and 106 people died. It was estimated that upto 6 mg aflatoxin B_1 per person was consumed.

2.8.6 Other species:

Wogan (1966) reported that hamsters are apparently sensitive to aflatoxin, but not as sensitive as duckling or the rat. The LD_{50} for hamsters is reported as 10.2 mg/kg body weight for aflatoxin B_1 .

Halver et al. (1966) reported that salmon were 10 to 20 times more resistant to acute aflatoxicosis than rainbow trout. They also reported LD_{50} of aflatoxin B_1 for salmon below 5 and 10 mg/kg body weight.

Chaffee et al. (1969) reported that aflatoxin contaminated corn caused aflatoxicosis in dogs.

Bountibonnes (1969) observed acute toxic symptoms in rainbow trout by intraperitoneal injection of aflatoxin

G_1 . The LD_{50} value was 1.90 mg/kg for G_1 while it was 0.81 mg/kg body weight for aflatoxin B_1 .

Chou et al. (1976) studied the susceptibility of mink to aflatoxin by giving a single dose of 300, 600 and 900 µg of aflatoxin/kg body weight. Minks died in three different doses were 1, 2 and 4 out of 5 minks in each group within 4 days due to acute aflatoxicosis.

Greene and Oehme (1976) reported the first case of acute equine aflatoxicosis in a 15 year old Arabian stallion in Kansas. The symptoms showed were of anorexia, loss in weight and jaundice. The horse feed was found to be mouldy and contained a very high level of aflatoxin B_1 .

It appears from the literature that more studies are needed in farm animals to elucidate the safe level of aflatoxins for different categories of livestock.

2.9 EFFECT OF AFLATOXINS ON NITROGEN METABOLISM

Lynch et al. (1970) carried out experiments to study the response of dairy calves to aflatoxin contaminated feed. Six aflatoxin dose levels from 0.08 to 0.8 mg/kg body weight were fed to six pairs of young dairy calves for six weeks. There was no significant effect on total serum proteins and albumin/globulin ratio as a result of aflatoxin feeding. In another study, Lynch et al. (1973) studied the effect of graded levels of aflatoxin ranging from 0 to 0.08 mg/kg body weight in young dairy calves on nitrogen balance. Nitrogen retention declined

significantly with increase in dose level. However, none of the calves showed negative nitrogen balance.

Hale and Wilson (1979) observed the effect of feeding aflatoxin contaminated maize on nitrogen metabolism in pigs. Pigs fed aflatoxin free diet consumed less nitrogen, excreted less nitrogen in urine and had a higher nitrogen balance than pigs fed diets with aflatoxin contaminated corn.

Neathery et al. (1980) fed dairy calves diets containing 40 to 650 ppm zinc with or without 5 ppm aflatoxins. The calves given aflatoxins had low nitrogen balance.

Osuna and Edds (1982) reported that in pigs given aflatoxin B_1, there was significant decrease in serum total proteins, α, β, γ globulins and fibrinogen but no effect on blood urea-N.

Maurice et al. (1983) observed that the feeding of aflatoxin B_1 at a level of 50 or 100 µg/kg body weight significantly decreased the plasma lysine and histidine and significantly increased plasma ceruloplasmin.

Burguera et al. (1983) found low values of total protein, albumin, globulin in turkey poults fed aflatoxin and crotolaria but the values of total protein, albumin and β globulin were higher in groups given aflatoxin plus selenium.

Murthy et al. (1984) studied the effect of feeding aflatoxin at a level of 10 or 70 µg/kg body weight to cattle and buffalo calves. They found decreased concentration

of serum proteins in the groups fed aflatoxin in both the species.

Wyatt et al. (1985) fed young male Holstein calves diets containing 40 or 640 ppm zinc with 0 or 5 ppm aflatoxin for 3 weeks. They did not observe any effect on plasma total protein, albumin, globulin and albumin/globulin ratio.

For better understanding, further research work is necessary to study the effect of aflatoxin toxicity on nitrogen dynamics in cattle and buffalo, using latest available techniques.

2.10 *TISSUE DISTRIBUTION AND EXCRETION PATTERN OF AFLATOXIN*

The aflatoxins are metabolized by the hepatic mixed function oxidase (MFO), to a group of hydroxylated derivatives which are species specific and are excreted through faeces, urine and milk in lactating animals.

Allcroft and Carnaghan (1963) were the first to detect aflatoxin M_1 in milk, principally with protein fraction. The quantity of aflatoxin excreted in milk is directly proportional to its intake. When high levels of aflatoxin are ingested, 1-3 per cent of the ingested dose appears in milk. Milk levels of aflatoxin drop rapidly when intake is discontinued (Allcroft and Roberts, 1968). Similar were the findings of Masri et al (1969) in lactating cows.

Shank and Wogan (1965) studied the distribution and excretion pattern of ^{14}C-aflatoxin following its oral

administration in rats. The radioactivity was 12.3, 7.0, 43.8, 20.7 and 5.7 per cent in carbondioxide, urine, stomach contents, intestinal contents and liver.

Nabney et al. (1967) conducted an experiment to study the excretion pattern of aflatoxin in sheep. They found that the principal route of the excretion of aflatoxin M_1 was through urine, while aflatoxin G was excreted both in the urine and faeces.

Mabee and Chipley (1973) observed the excretion and tissue distribution of ^{14}C-aflatoxin B_1 in broiler chickens. The broiler chickens excreted 90.64 per cent of the dose administered. The radioactivity distributed in various organs was 11.04, 0.84, 4.30, 12.52, 32.66 and 30.13 per cent in blood, liver, heart, gizzard, breast and leg respectively.

Polan et al. (1974) studied the excretion pattern of ^{3}H-aflatoxin B_1 in lactating cows. The labelled aflatoxin was given orally. Approximately one-half of the tritium excreted in urine over 96 hours occurred during the first 24 hours. In contrast to the excretion pattern in urine, the cumulative tritium content of milk, percentage wise was increasing at its fastest rate between 40 and 60 hour. After 96 hour, total labelled material excreted was less than 15 per cent of the labelled dose given orally. The unaccounted tritium was considered present in different body tissues.

Lafont and Lafont (1975) fed lactating cows on hay and groundnut cake with a mean content of aflatoxin B_1

about 85 mg/kg. An average of 0.95 per cent of the ingested aflatoxin was excreted through milk. Engel and Hagemeister (1978) reported that only 2 to 5 per cent of feed toxin reached the small intestine of cow and 0.6 per cent of the feed aflatoxin B_1 was excreted as hydroxy derivatives with milk.

Applebaum and Marth (1981) studied the excretion of aflatoxin B_1 into milk of lactating cows fed 13 mg of pure aflatoxin for 7 days. The conversion of aflatoxin B_1 to aflatoxin M_1 was not greater than 1 per cent. Aflatoxin M_1 was detected in milk 4 days post toxin administration.

Kong et al. (1982) observed the effect of feeding aflatoxin B_1 on aflatoxin M_1 excretion in milk of lactating cows. They found a highly significant correlation ($r=0.935$) between the toxin intake and excretion in milk. There was a wide variation in conversion of B_1 to M_1 which ranged from 3.45 to 11.39 per cent in different cows.

Trucksess et al. (1983) gave a single oral dose (0.5 mg/kg body weight) of aflatoxin to 2 lactating cows. Samples of milk, urine and faeces were collected at different time intervals for 10 days. Aflatoxicol (R_0), aflatoxins B_1 and M_1 were found in the milk, plasma and red blood corpuscles of both cows at post dosing hour 1. Maximum concentration of the toxin was observed at 12 and 60 hours of aflatoxin dosing. One cow died after 60 hours of aflatoxin dosing. The aflatoxin B_1 concentration in the samples of liver, kidney, urine, bile and rumen contents of the

dead cow were 5.1, 3.3, 4.1, 1.6 and 320 ng/g respectively and M_1 concentrations were 4.3, 20, 37, 16 and 86 ng/g.

Helferich et al. (1986) studied the excretion pattern of aflatoxin in lactating goats by giving ^{14}C-aflatoxin B_1 either orally or intravenously. Milk, faeces and urine were collected for 120 h after ^{14}C (Af-B_1) administration. Recoveries of ^{14}C in urine, milk and faeces were respectively 22.7, 0.97 and 65 per cent (i.v. dose) and 30.9, 1.05 and 52.3 per cent (oral dose).

The excretion and retention of aflatoxin in different body compartments of livestock carries significant importance because it can cause health hazard to human beings who depend solely for their animal protein requirement from milk, meat and eggs. Therefore, detailed studies are required to be conducted for its tissue distribution, retention and secretion in milk.

2.11 EFFECT OF AFLATOXINS ON RUMEN FERMENTATION

Fehr and Delage (1970) reported that aflatoxin B_1 led to decrease in volatile fatty acid production in mixed rumen culture under in vitro conditions. They also observed a decrease in acetic acid and increase in propionic acid concentration. Repetto (1976) also noticed that with the increase in the level of aflatoxin to artificial rumen, cellulolysis and production rate of VFA decreased.

Allcroft (1969) reported that mature ruminant was less susceptible than the young ruminant to the toxic effect of aflatoxin. This could be related to some detoxifying

ability of the microorganisms in the rumen of mature animal. But Mathur et al. (1976) indicated that concentration of aflatoxin B_1 in the sample before and after 24 hours incubation with rumen fluid was over 90 per cent of its original concentration and thus ruled out the possibility of degradation of aflatoxin in rumen. Contrary to this Engel and Hagemeister (1978) observed the presence of aflatoxin M_1 in the rumen sample which confirmed the transformation of aflatoxin B_1 in the rumen of dairy cows. In their in vitro experiments also, the results indicated 40 per cent degradation of aflatoxin after 24 hours incubation with rumen fluid.

Sinha and Arora(1982) observed a significant decrease in cellulose disappearance, VFA concentration and protein synthesis with the increase in the level of aflatoxin in the artificial media. In another study, Sinha and Arora (1984b) reported that the concentration of total volatile fatty acids and propionic acid in the rumen of kids given aflatoxin was significantly less than those not given aflatoxin.

Balaraman and Arora (1984) reported that addition of aflatoxin to the artificial media had no adverse effect on protein synthesis or cellulose digestion or volatile fatty acid production, when the level of protein was enhanced from 100 mg to 150 mg in the incubation vessel, indicating that protein has got a protective effect against aflatoxin action on microbes.

Now, it is rather well established that protein

has got a protective effect against aflatoxicosis. But since the availability and cost of protein rich concentrates is limiting, some alternates are needed to be explored.

2.12 MODIFYING EFFECTS OF DIETARY NUTRIENTS

The toxic effect of aflatoxin as a dietary contaminant depends among other factors, also by the nutritional status of the animal. The dietary nutrient, interact with aflatoxin during the process of metabolism modifying its toxic effect. The different nutrients have been found to interact differently. The knowledge of such interactions may help in alleviating the toxic effects of the toxin.

Madhavan and Gopalan(1965) observed that the toxicity of aflatoxin B_1 in rats was more with protein deficient diet. Adekunle et al. (1977) found that the conversion of aflatoxin B_1 to aflatoxin M_1 and aflatoxin Q_1 was reduced by 50 per cent in rats, when their diet was deficient in protein.

Smith et al. (1971) reported that chicks were protected against the adverse effects of 5 ppm dietary aflatoxin on growth when the protein level in the diet was increased from 20 to 30 per cent.

A decrease in hepatic aflatoxin B_1 macromolecule adduct formation was noted by Preston et al (1976) in rats fed protein deficient diet. Sinha and Arora (1984b) observed that pre-ruminant kids receiving 1 ppm aflatoxin in the diet had normal growth comparable to the control kids due to higher protein level in the diet.

Wells et al. (1974) reported that liver was more affected by aflatoxin when lard was the source of fat than when groundnut oil was used in the diet of rat. Though aflatoxicosis decreases the synthesis of fatty acids and causes impaired lipid transport, yet diets high in lipids have a mortality sparing effect against aflatoxicosis and the fat containing unsaturated fatty acids have a growth sparing effect (Hamilton, 1977).

Murthy et al. (1975) fed pigs on aflatoxin contaminated groundnut meal alongwith high carbohydrate diet and found that high amount of carbohydrate in the combined ration exerted a protective effect on the liver. Wise et al. (1978) reported that rats excreted less aflatoxin M_1 in the urine when they were fed on a sucrose rich diet as compared to rat fed on starch rich diet, inspite of the same level of aflatoxin in both the diets.

Hamilton and Garlich (1972) could not alter the fatty liver syndrome caused by aflatoxin by supplementing the diet with 13.2 µg/kg vitamin B_{12}. Temcharoen et al. (1978) observed an increase in hepatic carcinoma cases as a result of feeding vitamin B_{12} (50 ug/kg) to rats fed aflatoxicated diet.

Barber et al. (1968) observed that aflatoxin B_1 reduced the vitamin A content of the liver of pigs. Similar results were reported by Hamilton (1975) in cattle and chicks. Addition of vitamins A and E to the ration increased the growth rate of young rats but did not stop the development of histopathological changes, mortality or clinical

signs of aflatoxicosis (Todd et al., 1968). Reddy et al. (1973) reported that rats on a vitamin A deficient diet were more sensitive to a single dose of aflatoxin.

Hamilton et al. (1974) observed that a diet deficient in vitamin D_3 makes chicks sensitive to doses of aflatoxin, while thiamine deficiency had the opposite effect (Hamilton, 1977).

Neathery et al. (1980) could not find any beneficial effect of zinc supplementation in calves fed aflatoxin in the diet.

Selenium has been reported to have protective effect against aflatoxin toxicity, at lower levels in pig (Davila et al., 1983), turkeys (Burguera et al., 1983; Gregory and Edds, 1984) and calves (Burcato et al., 1986).

2.13 EFFECT ON IMMUNITY

Aflatoxin adversely effects immunity in animals by depressing antibody formation, by altering the interaction between the immunogen and host tissues and by reducing the formation of non-humoral substances associated with disease resistance (Pier, 1973).

Aflatoxin was reported to reduce the resistance of chicken to infection by Candida albicans (Hamilton and Harris, 1971). Edds et al. (1973) observed that chicken previously exposed to aflatoxin B_1 had higher susceptibility t Coccidiosis and infection by Marek's disease virus. However, aflatoxin at dose level of 15 µg per day for 7 days had no effect on antibody production against Salmonella pullorum

antigens in chicks (Adinarayanaiah et al., 1973).

Paul et al. (1977) reported that aflatoxin signifi - cantly suppressed the in vitro bovine lymphocyte response to phytomitogens. Sinha and Arora (1984b) observed a similar reduction in response to phytomitogens by lymphocytes in goats.

Southern and Clawson (1979) observed that aflatoxin at dietary levels of 750 ppb and above caused an increase in serum IgM fraction in pigs.

Eddy et al. (1980a) found that aflatoxin fed to Holstein heifers upto 4.8 mg per day for 140 days, did not affect the immune response to certain live virus preparations.

Visalakshan et al. (1984) found that T lymphocytes and total lymphocytes counts were reduced in pigs fed aflatoxin @ 0.15 mg/kg body weight for 3 months.

Thurston et al. (1986) reported that feeding of aflatoxin to cattle depressed both complement dependent and independent seruum bacteriostatic activity as measured by growth inhibition of Escherchia coli.

Rao (1987) attributed the persistance of New Castle disease in chicks to immuno-suppression caused by aflatoxin in feed.

Ilgaz et al. (1987) observed immunosuppression in chicks as a result of aflatoxin feeding.

2.14 AFLATOXIN RESIDUES IN FOODS OF ANIMAL ORIGIN

The mode of entry of aflatoxins in human food is

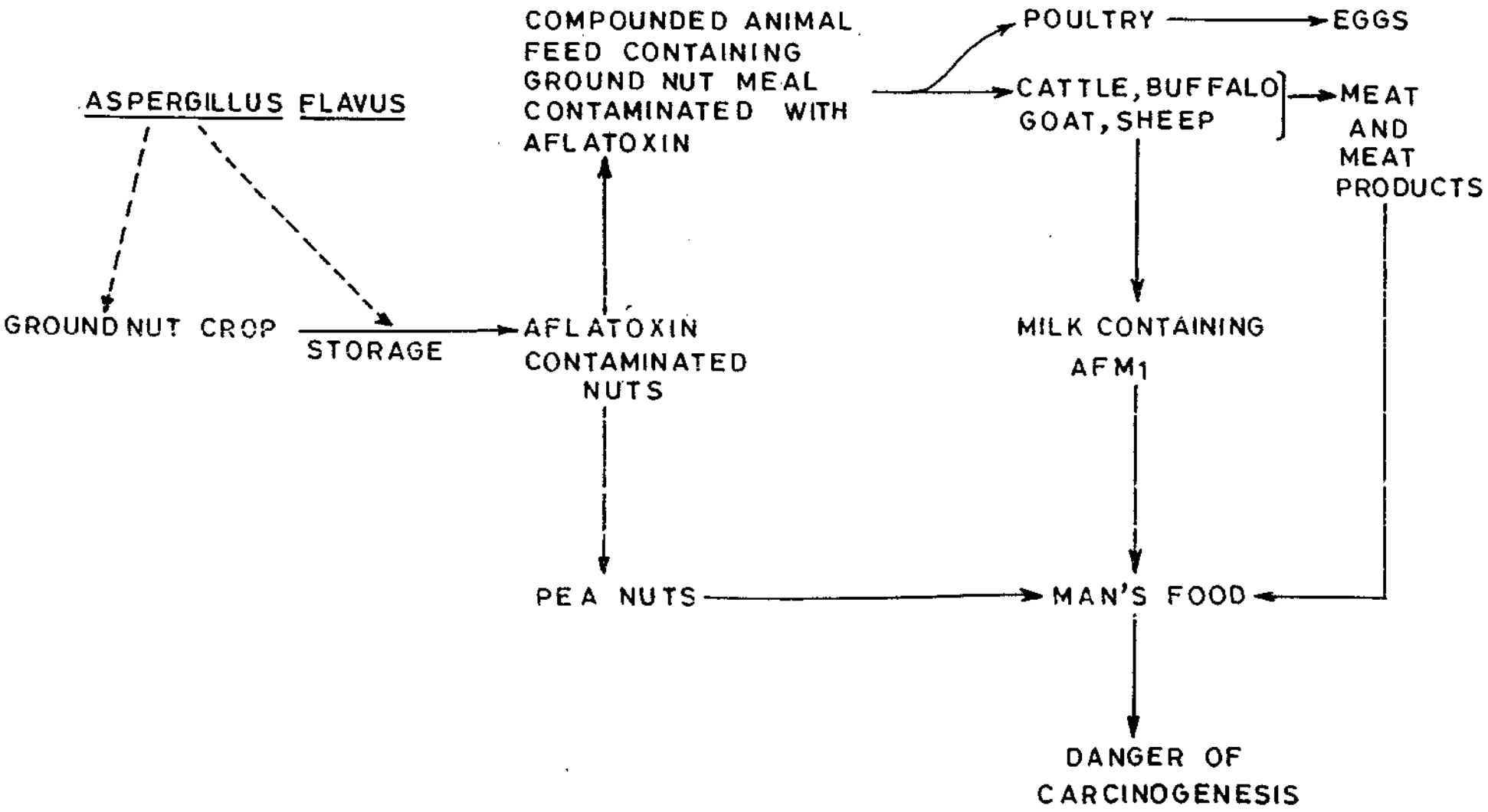
given in Fig. 4. The toxic metabolites that accumulate in milk and tissues of food producing animals pose serious problem to the human health.

Milk is most commonly found to be contaminated with aflatoxin M_1 . Allcroft and Roberts (1968) measured the amount of aflatoxin M_1 in milk from cows given diets containing various levels of aflatoxin. Results indicated that the amount of aflatoxin M_1 excreted in milk was in direct proportion to the intake of aflatoxin B_1 . Secretion of aflatoxin M_1 into milk as percentage of aflatoxin consumed varies from less than 1 to 3 per cent Linde et al., 1963; Masri et al., 1967; Nabney et al., 1967; Polan et al., 1974; Kong et al., 1982; Frobish et al., 1986).

Rodricks and Stoloff(1977) calculated that in general the ratios of the levels of aflatoxin B_1 in the feed to that in the milk were about 300:1; so that a compound feed containing 30 µg/kg of aflatoxin B_1 would be expected to contaminate milk with 0.1 µg/litre of aflatoxin M_1 . However, the conversion varies widely from animal to animal and the overall range of values is reported to vary between 34:1 and 1600:1 (Stoloff, 1980).

Allcroft and Carnaghan (1963) noticed that aflatoxin M_1 in milk of cows was mostly associated with the casein fraction but McKinney et al. (1973) observed that during rennet precipitation, curd and whey carried aflatoxin M_1 in the ratio of 80:20. Stoloff et al. (1975) also found that aflatoxin M was distributed both in the curd and whey fraction of milk. Survey in West Germany revealed the

FIG. 4. MODE OF ENTRY OF AFLATOXINS IN HUMAN FOOD



presence of aflatoxin M_1 in milk ranging from traces to 0.54 µg/kg (Polzhofer, 1977). The aflatoxin content was rather high in milk samples analysed in Iran (Suzanger et al., 1976).

Yadagiri and Tulpule (1974) reported the presence of aflatoxin M_1 ranging from traces to 4.8 µg/litre in buffalo milk samples from the Government Dairy Farm,Kakinada in Andhra Pradesh, India. Lembe (1977) surveyed the milk and milk products in and around Karnal, Haryana, India, for the presence of aflatoxin and detected aflatoxins in various concentration in milk, khoa and cheese samples.

Survey conducted in United States of America on commercial milk samples and various dairy products did not reveal the presence of aflatoxin M_1 (Stoloff et al., 1981), indicating that it was possible to avoid aflatoxin residues in milk and its products by preventive measures.

Occurrence of aflatoxin residues in different tissues of food animals have also been reported by dairy research workers (Keyl and Booth, 1971; Rodricks and Stoloff, 1976; Hayes et al., 1977).

Krogh et al. (1973) showed the presence of aflatoxin residues in liver, kidney, heart, muscle and adipose tissues of adult swine fed with aflatoxin contaminated rations.

Jarvis (1976) reported the presence of aflatoxin in liver, kidney and other tissues of pig in feeding trials with aflatoxin contaminated diets. Levels were highest in livers and kidneys with only traces occurring in heart, muscle and adipose tissues.

Stoloff (1977) detected aflatoxins in tissues of broiler chickens and eggs of laying hens given aflatoxin contaminated feed.

Van Egmond et al. (1979) reported that in a survey of aflatoxin levels in livers, from slaughter houses, a sample of bovine liver was found to contain aflatoxins B_1, B_2 and M_1 at levels of 0.10, 0.03 and 0.08 µg/kg respectively.

Stubblefield et al. (1983) found aflatoxin B_1 and M_1 in all samples, except the thymus of a cow given aflatoxin B_1 orally at 0.35 mg/kg body weight for 3 days and slaughtered after 24 hours of the final dose. Kidney, liver and mammary gland had the highest concentrations.

Aflatoxin contamination of fish and fish products has been reported in Philippines at an average level of 3, 2, 5 and 2 µg/kg in dried fish, fish sauce, smoked fish and salted and fermented small shrimp respectively (FAO, 1979). Shank et al. (1972) reported that the average aflatoxin content of dried fish shrimp sample was 166 µg/kg, while salty fish in Indonesia was found to contain 5 µg/kg (FAO, 1979).

Although the contamination of aflatoxin at varying levels has been reported in most of the food products, systematic surveys have been carried out only in few countries and mostly for selected commodities. Aflatoxin contamination may vary considerably from year to year as well as from region to region of a country.

CHAPTER 3

Experiment 1:

Effect of aflatoxin on *in vitro* rumen microbial activity as influenced by different levels of selenium

EFFECT OF AFLATOXIN ON IN VITRO RUMEN MICROBIAL ACTIVITY AS INFLUENCED BY DIFFERENT LEVELS OF SELENIUM

Aflatoxins are known for suppressing the rumen microbial activity (Mathur et al., 1976;Sinha and Arora, 1982). Higher levels of protein have been reported to have a check against this suppression (Balaraman and Arora, 1984). Recently, selenium at low levels has also been reported to have a protective effect against aflatoxicosis in pigs (Davila et al., 1983), turkeys (Burguera et al., 1983; Gregory and Edds, 1984) and calves (Burcato et al., 1986).

The purpose of the present study was to examine the protective effect of different selenium levels on rumen microbial activity against aflatoxin toxicity under in vitro conditions. The rumen microbial activity was estimated in terms of cellulose disappearance, total volatile fatty acids concentration, microbial protein synthesis and ^{35}S-incorporation into the microbial protein.

MATERIALS AND METHODS

3.1.1 Plan of the experiment:

The experiment was conducted in a 3 x 3 factorial design with three replicates in a complete randomised block design. Three levels of selenium, i.e. 0, 0.25 and 0.5 ppm were selected and each level was observed for its protective effect on rumen microbial activity against the adverse effect of three levels of aflatoxin, i.e. 0, 0.25 and 0.5 ppm, under in vitro conditions.

3.1.2 The in vitro system:

The in vitro systems were set up in 150 ml conical flasks fitted with tight rubber corks having Bunsen gas release valve (Bentley et al.,1954). McDougall's artificial saliva was used as the buffer in the incubation medium (McDougall, 1948). Pure cotton cellulose and glucose served as the carbohydrate substrates. Urea and ammonium sulphate provided the nitrogen and sulphur in the ratio of 10:1 for optimum growth and multiplication of rumen microbes (Arora et al., 1977). $Na_2{}^{35}SO_4$, carrier free, radioisotope obtained from the Bhabha Atomic Research Centre, Bombay was used in the medium to study the incorporation of sulphur into the microbial protein. To each incubation flask, 20 ml strained rumen liquor was added as the inoculum for microbial activity. The details of the in vitro system and different treatments are given in Table 4.

3.1.3 Management and feeding of donor animal for rumen liquor

A male buffalo calf, aged 3 years and weighing about 350 kg was operated to make a fistula in the rumen, which was fitted with a permanent cannula. This animal served as the donor of rumen liquor. The animal was housed individually and was offered daily with 3 kg of concentrate mixture and 20 kg of green maize. The concentrate mixture consisted of 40 parts maize, 32 parts groundnut cake, 25 parts wheat bran and 3 parts mineral mixture including salt. Its calculated nutritive value was 20.50% CP and 70.65% TDN. The feed was offered once at 9.00 A.M. and the animal had

Table 4. Details of *in vitro* system and particulars of different treatments

Attribute	TREATMENT									
	Zero hr (control)	24 hr (control)	Af 0 Se .25 (ppm)	Af 0 Se .5 (ppm)	Af .25 Se 0 (ppm)	Af .25 Se .25 (ppm)	Af .25 Se .50 (ppm)	Af .50 Se 0 (ppm)	Af .50 Se .25 (ppm)	Af .50 Se .50 (ppm)
	Treatment number									
	0	1	2	3	4	5	6	7	8	9
McDougall's buffer (ml)	10	10	10	10	10	10	10	10	10	10
Cellulose (mg)	500	500	500	500	500	500	500	500	500	500
Glucose (mg)[a]	50	50	50	50	50	50	50	50	50	50
Urea (mg)[b]	32	32	32	32	32	32	32	32	32	32
Ammonium sulphate (mg)[c]	6	6	6	6	6	6	6	6	6	6
$Na_2{}^{35}SO_4$ (ml)[d]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SRL (ml)	20	20	20	20	20	20	20	20	20	20
Distilled water (ml)	9.9	9.9	9.8	9.7	9.8	9.7	9.6	9.7	9.6	9.5
Aflatoxin (µg)[e]	-	-	-	-	0.2	0.2	0.2	0.4	0.4	0.4
Selenium (µg)[e] as sod. selenite	-		0.2	0.4	-	0.2	0.4	-	0.2	0.4

a = 50 mg/ml; b = 32 mg/ml; c = 6 mg/ml; d = 0.45 µCi/0.1 ml; e = 0.2 µg/0.1 ml

free access to clean water twice daily. The animal was adapted on this diet for 25 days before the collection of rumen liquor for the in vitro experiment.

3.1.4 Inoculum preparation and incubation:

Rumen liquor was collected at 9.00 A.M. before the scheduled feeding and watering with the help of a polythene tube of 1 cm diameter. The polythene tube was inserted approximately 30 cm deep into the rumen and rumen liquor was initially drawn with the help of a 50 ml plastic syringe and then syphoned off in a smooth flow. The liquor was collected and transported to the laboratory in a pre-warmed thermos flask and strained through four layers of muslin cloth. Twenty ml of strained rumen liquor was transferred into each incubation flask containing the substrates and was flushed with carbondioxide for 2 minutes to ensure anaerobic conditions and closed with rubber bung having Bunsen gas release valve. The flasks were incubated for 24 hours in a waterbath maintained at 39° ± 1°C with an automatic shaking arrangement. Zero hour controls were maintained to record the initial stage of microbial activity

3.1.5 Analytical procedures:

The zero hour controls were stopped for microbial activity with 10 ml of 1.07 N sulphuric acid and 10 ml of 10% sodium tungstate solution to estimate initial protein, cellulose and total volatile fatty acid concentration. Other flasks were incubated for 24 hours at 39° ± 1°C and at the end of incubation period, the microbial activity

was stopped. After 6 hours, the contents from each flask were quantitatively transferred to a polythene tube and centrifuged at 3,000 rpm for 20 minutes in a centrifuge. The supernatant was transferred to a 100 ml volumetric flask. The protein precipitate alongwith residual cellulose was washed thrice with 20 ml water, 5 ml of 1.07 N sulphuric acid and 5 ml of 10% sodium tungstate solution and centrifuged. The supernatant was again transferred to the same volumetric flask and the volume was made upto 100 ml mark and kept for the estimation of total volatile fatty acids. The residue was treated thrice with 15 ml of 0.1% sodium hydroxide with thorough mixing each time for one hour and then centrifuged at 3,000 rpm for 20 minutes each time to separate cellulose, and other feed particles. The supernatant containing dissolved protein was transferred every time to a 50 ml volumetric flask. The volume was made upto mark with 0.1N sodium hydroxide and kept for determination of protein ^{35}S radioactivity. The residue in the centrifuge tube was then transferred to a 50 ml glass tube for cellulose determination.

3.1.6 Analytical methods:

3.1.6.1 Cellulose estimation:

Cellulose in the residue was determined by the method of Crampton and Maynard (1938). Percent digestibility of cellulose was calculated on the basis of cellulose content at zero hour control.

3.1.6.2 Total volatile fatty acids:

Total volatile fatty acids (TVFA) were determined

by the method of Barnett and Reid (1957).

3.1.6.3 Microbial protein:

After incubation, protein was precipitated by the addition of 10% sodium tungstate solution (Shultz and Shultz, 1970) and was determined by the method of Lowry et al. (1951).

3.1.6.4 Radioactivity measurement for determining ^{35}S -incorporation into microbial protein:

Samples of standard, protein fraction and supernatants were prepared for radioactivity measurement by the method of Mahin and Lofberg (1966). The ^{35}S radioactivity measurements were carried out in PACKARD TRI-CARB PRIAS liquid scintillation counter (Model BPLDS No.00099) at 20°C. Net ^{35}S incorporation was arrived at by subtracting the value obtained from zero hour control from the gross ^{35}S incorporation values obtained from different treatments.

3.1.6.5 Statistical analysis of the data:

The data were analysed as per Snedecor and Cochran (1967).

3.1.7 Results and discussion:

The results on the effect of aflatoxin on cellulose digestion, total volatile fatty acids concentration, microbial protein synthesis and ^{35}S incorporation and on percent inhibition are presented in Tables 5 and 6 respectively. The analysis of variance of the data is presented in Table 7.

3.1.7.1 Cellulose digestion:

The result on cellulose digestibility at different

Table 5. Effect of different levels of aflatoxin and Selenium on cellulose disappearance, total volatile fatty acids concentration, microbial protein synthesis and ^{35}S incorporation*

Treatment No.	Treatment		Cellulose disappearance (%)	TVFA concentration (mM/100 ml SRL)	Net microbial protein synthesis (mg)	^{35}S incorporation (%)
	Af (ppm)	Se (ppm)				
1 (24 h control)	0	0	31.64 ± 1.89	2.18 ± 0.12	20.99 ± 1.81	30.65 ± 1.90
2	0	.25	30.91 ± 2.09	2.20 ± 0.60	19.53 ± 0.78	29.90 ± 2.10
3	0	.50	30.42 ± 1.67	2.20 ± 0.14	20.99 ± 1.11	29.40 ± 1.70
4	.25	0	21.17 ± 2.42	1.08 ± 0.23	9.84 ± 2.16	20.16 ± 2.43
5	.25	.25	16.87 ± 2.70	1.06 ± 0.24	6.93 ± 2.71	15.86 ± 2.70
6	.25	.50	17.62 ± 3.78	1.07 ± 0.37	9.57 ± 2.54	16.62 ± 3.78
7	.50	0	20.66 ± 3.57	1.11 ± 0.15	7.59 ± 1.57	19.65 ± 3.57
8	.50	.25	12.32 ± 1.67	0.81 ± 0.33	3.59 ± 0.77	11.31 ± 1.67
9	.50	.50	15.48 ± 3.51	0.33 ± 0.17	3.60 ± 1.06	14.47 ± 3.51
C.D.			4.28	0.41	3.07	4.26

* Each value is an average of 3 replicates

selenium levels without aflatoxin were T_1 :(control) 31.64 ± 1.89%; T_2 :30.91 ± 2.09%; T_3 : 30.42 ± 1.67%. These results indicated that there was no effect of these levels of selenium on cellulose digestibility under *in vitro* conditions. Little *et al*. (1958) had also earlier reported that cellulose digestion was not inhibited at such low levels of selenium. The results on cellulose digestibility at different levels of aflatoxin, alongwith different levels of selenium were: T_4 : 21.7 ± 2.42%; T_5 : 16.87 ± 2.70%; T_6 : 17.62 ± 3.78%; T_7 : 20.66 ± 3.57%; T_8 : 12.32 ± 1.67%; T_9 : 15.48 ± 3.51% and the percentage inhibition in cellulose disappearance was 33.09, 46 68, 44.31,34.70, 61.06 and 51.07 with these treatments respectively. Statistical analysis of the data indicated that aflatoxin toxicity at different levels led to significant decrease ($P \leq 0.01$) in cellulose digestibility (%) showing adverse effect on cellulolytic microorganisms. Further, it was evident that the levels of selenium used in this study did not protect the microbes.

3.1.7.2 Total volatile fatty acids concentration:

The results on TVFA concentration (mM/100 ml SRL) at different levels of selenium without aflatoxin were: T_1 : 2.18 ± 0.12; T_2 : 2.2 ± 0.60; T_3 : 2.2 ± 0.14 indicating no effect. The results on TVFA concentration(mM/100 ml SRL) at different levels of aflatoxin with varying levels of selenium were: T_4 : 1.08 ± 0.23; T_5 : 1.06 ± 0.24; T_6 : 1.07 ± 0.37; T_7 : 1.11 ± 0.15; T_8 : 0.81 ± 0.33; T_9 : 0.33 ± 0.17. The percent inhibition was 50.46, 51.38, 50.92, 49.08, 62.84 and 84.86 respectively with the above treatments

Table 6. Percent inhibition in cellulose disappearance, total volatile fatty acids concentration, microbial protein synthesis and ^{35}S incorporation

Treatment No.	Treatment		Cellulose dis-appearance	TVFA concentra-tion	Net microbial protein synthesis	^{35}S-incorporation
	Af (ppm)	Se (ppm)				
1 (Control)	0	0	0	0	0	0
2	0	.25	2.31	0	6.96	2.45
3	0	.50	3.86	0	0	4.08
4	.25	0	33.09	50.46	53.12	34.23
5	.25	.25	46.68	51.38	66.98	48.25
6	.25	.50	44.31	50.92	54.41	45.77
7	.50	0	34.70	49.08	63.84	35.89
8	.50	.25	61.06	62.84	82.90	63.10
9	.50	.50	51.07	84.86	82.85	52.79

with different levels of aflatoxin. The data revealed that there was a significant decrease ($P < 0.01$) in total volatile fatty acids concentration with the increase in the level of aflatoxin at all levels of selenium. Sinha and Arora (1982) and Arora et al. (1988c) also reported a decrease with the increase in aflatoxin level under in vitro conditions. Selenium levels used in this study could not check the suppression of microbial activity as a result of different aflatoxin levels.

3.1.7.3 Protein synthesis:

The results on net microbial protein synthesis at different selenium levels without aflatoxin were: T_1 (control) 20.99 ± 1.81 mg; T_2: 19.53 ± 0.78 mg and T_3: 20.99 ± 1.11 mg. These levels of selenium had no effect on microbial protein synthesis. Khirwar and Arora (1976) reported that selenium levels higher than 30 µg (1.0 ppm) were detrimental to the growth of microorganisms. The results on net microbial protein synthesis at different levels of aflatoxin with or without selenium were: T_4: 9.84 ± 2.16 mg; T_5: 6.93 ± 2.71 mg; T_6: 9.57 ± 2.54 mg; T_7: 7.59 ± 1.57 mg; T_8: 3.59 ± 0.77 mg and T_9: 3.60 ± 1.06 mg. The corresponding percent inhibition with different levels of aflatoxin and selenium was 53.12, 66.98, 54.41, 63.84, 82.90 and 82.85 respectively, indicating a significant decline ($P < 0.01$) with all the treatments with aflatoxin. Balaraman and Arora (1984) also observed less protein synthesis containing aflatoxin in the media. It was also apparent that all the levels of selenium were ineffective in checking the antimicrobial action of aflatoxin in this study.

Table 7. Analysis of variance

Source	d.f.	TVFA		Cellulose dis-appearance		Net microbial protein synthesis		^{35}S- incorporation	
		MSS	F	MSS	F	MSS	F	MSS	F
Between replicates	2	0.192	1.14	51.70	2.82	2.55	0.27	51.83	2.80
Between Selenium level	2	0.078	0.47	48.29	2.64	14.09	1.40	48.49	2.65
Between aflatoxin level	2	5.69	33.99**	570.74	31.14**	586.21	62.19**	571.22	31.77**
Interaction	4	0.08	0.48	18.33	0.59	3.78	0.40	10.87	0.594
Error	16	0.167		10.00		9.43			
Total	26								

** $P < 0.01$

3.1.7.4 ^{35}S incorporation into microbial protein:

The results on ^{35}S-incorporation into microbial protein at different selenium levels without aflatoxin were: T_1 (control) 30.65 ± 1.90%; T_2 : 29.90 ± 2.10% and T_3 : 29.40 ± 1.70%, which were not different statistically, indicating that selenium at these three levels in the artificial rumen fermentation study did not affect the ^{35}S-incorporation into microbial protein. Khirwar and Arora (1976) reported that overall ^{35}S-incorporation was uniform in protein fraction of all the treatments upto 30 µg (1.0 ppm) selenium level. The results on ^{35}S-incorporation into microbial protein fraction at different levels of aflatoxin with varying levels of selenium were T_4: 20.16 ± 2.43%; T_5: 15.86 ± 2.70%; T_6: 16.62 ± 3.78%; T_7: 19.65 ± 3.57%; T_8: 11.31 ± 1.67% and T_9: 14.47 ± 3.51%. The percent inhibition on ^{35}S-incorporation with different doses of aflatoxin and selenium was 34.23, 48.25, 45.77, 35.89, 63.10 and 52.79 respectively in treatments T_4 to T_9. A significant depression ($P < 0.01$) on ^{35}S-uptake by the microbes might be due to the bacteriocidal action of the aflatoxin. Different levels of selenium used in this study could not overcome the adverse effect of aflatoxin as observed with other parameters. Decrease in ^{35}S-incorporation into microbial protein fraction, under conditions similar to this study have even earlier been reported by Sinha and Arora (1982).

The protective effect of selenium at low levels against toxicity reported in rats (Newberne and Conner,

1974), swine (Davila et al., 1983) and turkeys (Burguera et al., 1983; Gregory and Edds, 1984) could not be confirmed on rumen microbes in this study. Selenium probably exerted its beneficial effect in above studies through glutathione peroxidase by protecting the hepatic cels against the toxic effect of aflatoxin (Davila et al., 1983). In this study, the anti-microbial activity of aflatoxin might have an upper hand and impaired the multiplication of celluolytic as well as proteolytic microorganisms before selenium could exerts its beneficial effect. Earlier such an inhibitory action has also been shown on Lactobacillus bulgaricus 1377 (Lembe, 1977), Flavobacterium aurantiacum (Lillehoj et al., 1967) and E. coli (Wragg et al., 1967) when grown in presence of aflatoxin B_1, as a result of less activity of DNA polymerase. Mathur et al. (1976) also reported inhibition in the growth of various microbes due to aflatoxin under in vitro conditions.

CHAPTER 4

Experiment 2:

Effects of aflatoxin on growth in kids and its influence on feed conversion efficiency

EFFECTS OF AFLATOXIN ON GROWTH IN KIDS AND ITS INFLUENCE ON FEED CONVERSION EFFICIENCY

Adverse effects of aflatoxins in various species are well documented. Young animals are more prone to aflatoxicosis than the old animals. In the present study, rate of growth was taken as a criterion for assessing the safe level of aflatoxins in the diet of crossbred kids fed graded levels.

MATERIALS AND METHODS

4.1.1 Plan of the experiment:

Twenty-four growing male kids of Alpine, Beetal and Saanen crosses were used in this study. They were randomly distributed into 4 groups of six kids each. All the animals were fed concentrate mixture and lucerne fodder as per their requirements. In addition, animals of groups 1, 2, 3 and 4 were given aflatoxin at the levels of 0, 0.3, 0.6 and 1.0 ppm respectively. All the animals were weighed weekly to assess their growth rate. Daily dry matter intake was also recorded to know the feed conversion efficiency in different groups. This feeding practice lasted for 16 weeks. The particulars of kids and their distribution in different groups are presented in Table 8.

4.1.2 Housing and management of kids:

Kids were kept individually in pens of size 1.5 m x 2.0 m. The pens were thoroughly white washed and disinfected before the start of the experiment. Disinfection was carried

Table 8. Particulars of kids and their distribution

Treatment group	Kid No.	Date of birth	Birth weight (kg)	Age at the start of experiment (days)	Initial body weight (kg)
	AB 674	12.11.86	3.4	140	10.2
Group 1	AB 678	14.11.86	3.6	138	11.0
0 ppm Af	AB 681	17.11.86	2.8	135	11.2
(control)	AB 689	24.11.86	3.2	127	11.8
	ASB 419	15.11.86	4.2	137	11.0
	SB 297	13.11.86	2.8	139	12.2
Average ± S.E.				136 ± 1.93	11.23±0.26
	AB 676	13.11.86	2.5	139	10.6
	AB 683	18.11.86	4.0	134	11.0
Group 2	AB 692	7.12.86	3.2	114	11.0
0.3 ppm Af	SB 301	21.11.86	3.2	132	11.0
	SB 420	15.11.86	3.5	137	11.6
	SB 425	25.11.86	3.4	127	11.2
Average ± S.E.				130.5± 3.72	11.07±0.12
	AB 680	16.11.86	4.0	136	11.0
	AB 688	24.11.86	3.5	128	12.4
Group 3	AB 691	6.12.86	2.2	115	10.0
0.6 ppm Af	SB 298	13.11.86	2.6	139	10.8
	ASB 418	14.11.86	3.2	138	11.0
	ASB 424	21.11.86	2.8	132	11.0
Average ± S.E.				131.3±3.67	11.0±0.29
	AB 677	14.11.86	4.0	138	10.8
Group 4	AB 685	19.11.86	5.4	133	11.2
1.0 ppm Af	AB 693	10.12.86	3.0	111	9.2
	SB 296	12.11.86	3.0	140	10.8
	ASB 423	20.11.86	3.2	132	11.2
	ASB 426	25.11.86	3.2	127	13.2
Average ± S.E.				130.17±4.25	11.07±0.48

out daily in the morning at the time of cleaning of the pens and white washing was repeated every month during the experimental period. The kids were given an adaptation period to their surroundings, management routine and their feeding regimen for about 2 weeks before starting the experiment.

4.1.3 Feeding schedule:

NRC recommendations (1981) for feeding of dairy goats were adopted for developing the feeding regimen. The kids were fed concentrate mixture and lucerne fodder according to the feeding schedule given in Table 9. The concentrate mixture was prepared by using different quantities of maize, wheat bran and mineral mixture (Table 10). Weighed quantity of concentrate mixture and lucerne fodder was offered at 9.30 A.M. and 12.30 P.M. respectively. Requisite quantity of aflatoxin was fed manually to each kid from surety stand point. Clean drinking water was provided ad lib to kids twice daily.

4.1.4 Recording of weight gain and dry matter feed intake:

The kids were weighed at weekly intervals prior to feeding and watering in the morning. The dry matter of the concentrate mixture and green lucerne offered to the kids was determined. Left overs were collected at the end of 24 hours and weighed, and their dry matter was also determined for calculating the DM intake. Feed efficiency was expressed as feed required per unit weight gain.

4.2 RESULTS AND DISCUSSION

Initial body weights (kg) of kids in different

Table 9. Feeding schedule

Body weight (kg)	Concentrate mixture		Lucerne fodder		Total		
	FM (g)	DM (g)	FM (kg)	DM (g)	DM (g)	CP (g)	TDN (g)
9 - 10	175	157.5	1.25	187.5	345.0	47.2	249.3
11 - 12	200	180.0	1.50	225.0	405.0	55.7	292.5
13 - 14	225	202.5	1.75	262.5	465.0	64.5	335.7
15 - 16	250	225.0	2.0	300.0	525.0	73.0	378.9
17 - 18	275	247.5	2.25	337.5	585.0	82.5	422.3
19 - 20	300	270.0	2.50	375.0	645.0	89.0	465.8
21 - 22	325	292.5	2.75	412.5	705.0	97.5	509.3
23 - 24	350	315.0	3.0	450.0	765.0	106.0	552.8
25 - 26	375	337.5	3.25	487.5	825.0	114.5	596.3
27 - 28	400	360.0	3.50	525.0	885.0	123.0	639.8
29 - 30	425	382.5	3.75	562.5	945.0	131.5	683.3

Table 10. Percent ingredient composition of concentrate mixture

Ingredient	Parts	CP	TDN
Maize	80	8.00	68.0
Wheat bran	17	2.13	1.0
Mineral mixture*	3	-	-
Total	100	10.13	79.0

* Composition of mineral mixture:

Ingredient	g per 3 kg mineral mixture
Dicalcium phosphate	1.6500
Sodium chloride	0.9000
Calcium carbonate	0.3312
Magnesium carbonate	0.0900
Ferrous sulphate	0.0150
Zinc sulphate	0.0075
Copper sulphate	0.0021
Manganese dioxide	.0021
Cobalt chloride	.0015
Potassium iodide	.0003
Sodium fluoride	.0003

treatment groups were: Group 1: 11.23 ± 0.26; Group 2: 11.07 ± 0.12; Group 3: 11.00 ± 0.29 and Group 4: 11.07 ± 0.48. They did not vary significantly amongst the various treatment groups. The average weekly weight gains (g) of kids were: Group 1: 508.50 ± 41.40; Group 2: 408.50 ± 43.40; Group 3: 330.10 ± 60.50 and Group 4: 228.60 ± 49.70, indicating the decline in weight gain with increase in aflatoxin levels. The cumulative weekly weight gains (kg) of kids in different treatment groups are given in Table 11 and depicted in Fig. 5. The total weight gains (kg) during the 16 week growth period were: Group 1: 8.14 ± 0.66; Group 2: 6.53 ± 0.69; Group 3: 5.28 ± 0.77 and Group 4: 3.66 ± 0.80. The average daily gains (g) of kids in different treatment groups were: Group 1: 72.33 ± 6.43; Group 2: 58.36 ± 6.79; Group 3: 47.15 ± 6.43 and Group 4: 32.66 ± 7.65. Regression analysis (Table 12) of weight gains of kids during 16 weeks gave the 'b' values (kg) per week as follows: Group 1: 0.51 ± 0.04; Group 2: 0.42 ± 0.04; Group 3: 0.30 ± 0.05 and Group 4: 0.21 ± 0.05, indicating a progressive reduction with incremental increase in the level of aflatoxin feeding.

The statistical analysis of the data indicated a significant decrease ($P < 0.01$) in growth rate in groups 3 and 4, while the decrease was non-significant in group 2, when compared to control group. Statistical analysis of the data also revealed that feeding of aflatoxin had no adverse effects on growth in any group upto 11 weeks. From 12 weeks onwards, the kids of group 4 showed a

FIG. 5. CUMULATIVE WEEKLY WEIGHT GAINS OF KIDS IN DIFFERENT GROUPS

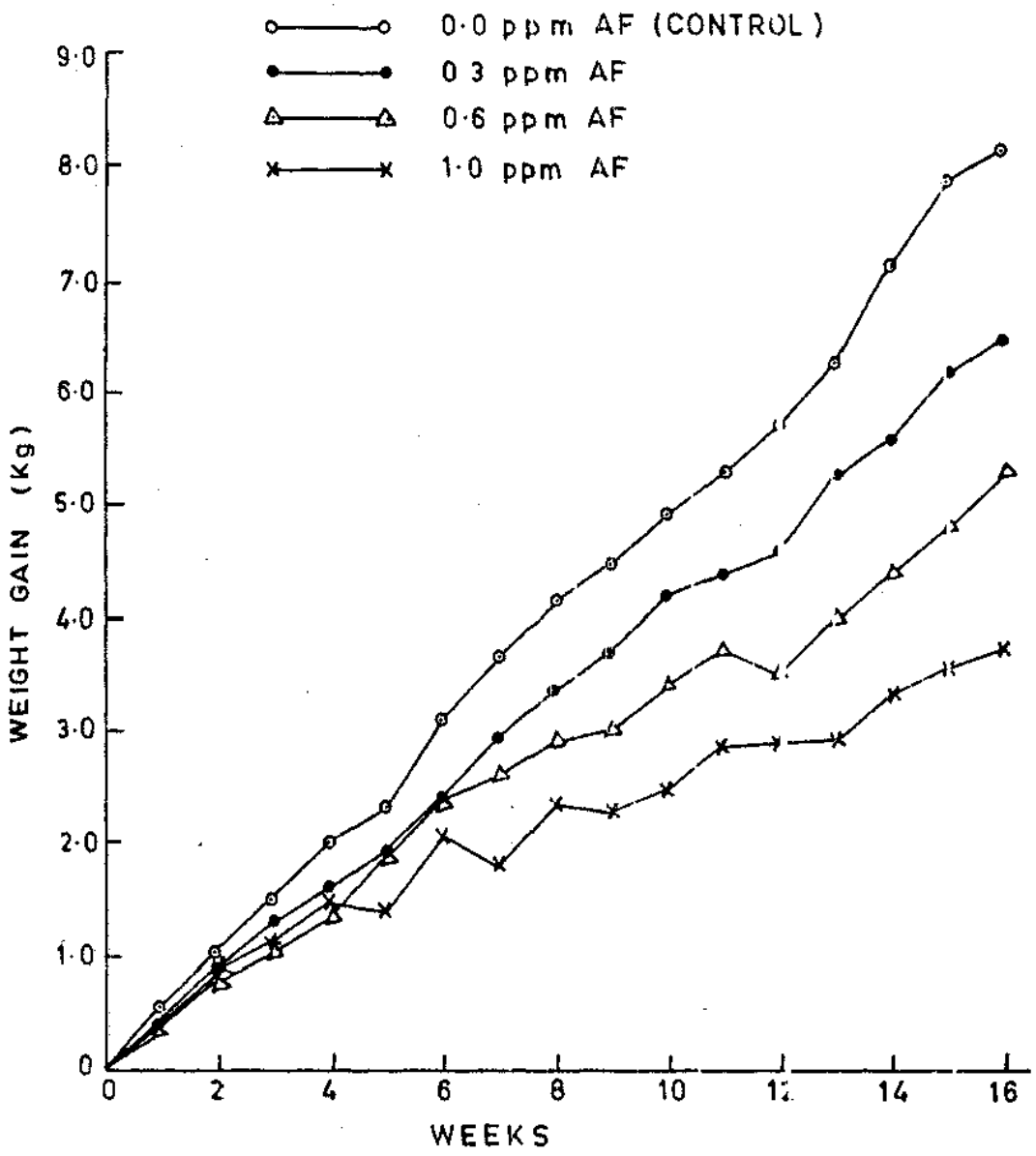


Table 11. Cumulative weekly weight gain in kids

Weeks	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
1	0.52±0.13	0.43±0.06	0.37±0.06	0.40±0.04
2	1.02±0.20	0.92±0.15	0.90±0.09	0.82±0.16
3	1.48±0.35	1.28±0.23	1.05±0.26	1.05±0.29
4	2.00±0.43	1.60±0.27	1.35±0.37	1.40±0.38
5	2.33±0.54	1.90±0.32	1.93±0.53	1.35±0.50
6	3.13±0.60	2.40±0.48	2.45±0.45	2.06±0.50
7	3.68±0.63	2.96±0.41	2.58±0.45	1.77±0.63
8	4.17±0.62	3.38±0.40	2.90±0.60	2.33±0.71
9	4.43±0.70	3.70±0.55	3.00±0.56	2.28±0.65
10	4.93±0.66	4.20±0.66	3.40±0.56	2.46±0.70
11	5.55±0.68	4.37±0.56	3.73±0.58	2.87±0.65
12	5.72±0.67	4.60±0.50	3.47±0.64	*2.80±0.77
13	6.56±0.63	5.30±0.77	*4.00±0.64	*2.92±0.73
14	7.17±0.82	5.60±0.74	*4.38±0.81	*3.33±0.87
15	7.85±0.75	6.20±0.77	*4.78±0.80	*3.57±0.98
16	8.14±0.72	6.53±0.76	**5.28±0.12	**3.67±1.01

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

Table 12. Effect of graded levels of aflatoxin on growth rate in kids

Attributes	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
Initial weight (kg)	11.23 ± 0.26	11.07 ± 0.12	11.00 ± 0.29	11.07 ± 0.48
Final weight (kg)	19.37 ± 0.84	17.60 ± 0.67	**16.28 ± 0.86	**14.73 ± 0.91
Weight gain (kg)	8.14 ± 0.66	6.53 ± 0.69	5.28 ± 0.77	3.66 ± 0.80
Weight gain/week (g)	508.50 ±41.40	408.50 ±43.40	**330.10 ±60.50	**228.70 ±49.70
Weight gain/day (g)	72.631 6.43	58.36 ± 6.79	**47.15 ± 6.43	**32.66 ± 7.65
Regression (b) value/week (kg)	0.51 ± 0.04	0.42 ± 0.04	** 0.30 ± 0.05	** 0.21 ± 0.05

** $P < 0.01$

depression in weight gain ($P < 0.05$) while the adverse effect of aflatoxin started from 13 weeks onwards in group 3. But Balaraman and Arora (1986) observed the retardation of growth in crossbred calves fed 1.0 ppm aflatoxin from 3rd week onwards. It might be due to the variation in susceptibility of different species to aflatoxin.

In the present investigation although there was a depression in growth rate of kids at 0.3 ppm level of aflatoxin, but statistically it was not signifcantly different from control group. Similar results were reported by Maryamma and Sivadas (1975) who found no adverse effect on growth in goats fed 0.4 ppm aflatoxin in their diet. Growth rate was significantly less in kids of groups 3 and 4, given 0.6 and 1.0 ppm levels respectively. Earlier, Arora et al. (1988b) had reported a significant decrease in growth rate in kids at aflatoxin levels ranging from 0.5 to 1.0 ppm. Thus, it may be inferred that even 0.3 ppm level of aflatoxin may not be tolerable to kids.

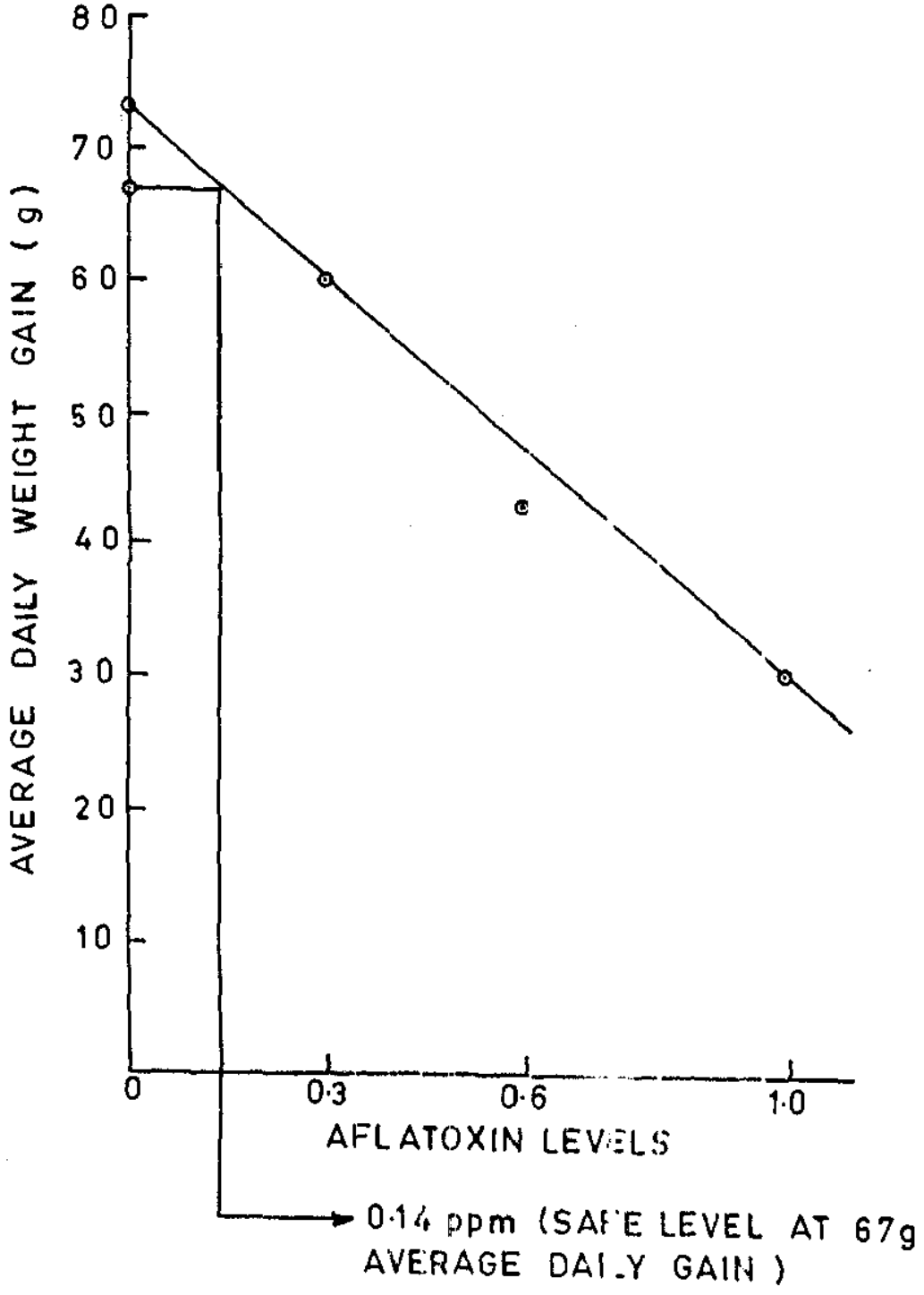
Details of dry matter intake and feed to gain ratios in different treatment groups are given in Table 13. Total dry matter consumption (kg) and average daily dry matter consumption (kg) in different treatment groups were: Group 1: 72.78 ± 2.52 and 0.650 ± 0.024; Group 2: 68.43 ± 2.33 and 0.611 ± 0.023; Group 3: 66.58 ± 2.67 and 0.594 ± 0.026 and Group 4: 63.85 ± 5.01 and 0.570 ± 0.040. Statistical analysis of the data did not reveal any difference as reported earlier (Sinha and Arora, 1984b) Feed to gain ratios were 8.95 ± 0.40; 10.48 ± 0.83; 12.60± 1.64 and 17.39 ±

Table 13. Dry matter consumption and feed to gain ratio

Attribute	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
Total DM consumption (kg)	72.78 ± 2.52	68.43 ± 2.33	66.58 ± 2.67	63.85 ± 5.01
Average daily DM consumption (kg)	0.650±0.024	0.611 ±0.023	0.594 ±0.026	0.570 ±0.040
Feed:gain ratio	8.96 ± 0.40	10.48 ± 0.83	**12.60 ± 1.64	**17.39 ± 3.66

** $P < 0.01$

FIG. 6. REGRESSION LINE FOR AVERAGE DAILY GAINS IN DIFFERENT GROUPS OF KIDS



3.66 in groups 1, 2, 3 and 4 respectively, indicating higher dry matter consumption per unit weight gain with increase in aflatoxin levels. These findings were in accordance with the earlier reports on feed : gain ratios (Sinha and Arora, 1984b; Balaraman and Arora, 1986; Arora et al., 1988b).

In the present study, effect of aflatoxin on growth was considered as the basis of assessing the safe level for kids and thus a regression line ($P < 0.01$) was drawn (Fig. 6) depicting the growth rate in different groups against graded levels of aflatoxin. On the basis of this regression line, it was inferred that 0.14 ppm might be the safe level for growth in the kids, with developed rumen, which confirmed the earlier finding (Arora et al., 1988b) where a safe level of aflatoxin was observed to be 0.147 ppm for pre-ruminant kids.

CHAPTER 5

Experiment 3:

Effects of aflatoxin on immunoglobulin status of blood and growth in buffalo calves

EFFECTS OF AFLATOXIN ON IMMUNOGLOBULIN STATUS OF BLOOD AND GROWTH IN BUFFALO CALVES

Aflatoxins have been reported to exert less resistance to infection and development of immunity (Pier, 1973). Reduced resistance to infection by *Candida albicans*, *Eimeria tenella* and Marek's disease virus was observed in poultry due to aflatoxin intake (Hamilton and Harris, 1971; Edds *et al.*, 1973). Aflatoxins are also known for the suppression of bovine and caprine lymphocyte response to phytomitogens under *in vitro* conditions (Paul *et al.*, 1977; Sinha and Arora, 1984b). Reduced passive immunity which was correlated with the low level of immunoglobulin in blood serum of neonatal calves was also due to aflatoxin intake (Balaraman and Arora, 1987). These adverse effects of aflatoxins vary from species to species and further it depends upon duration and amount of aflatoxin ingested and nutritional status of the animal.

The objective of this experimert was to study the effects of graded levels of aflatoxin on immunoglobulin status in blood serum and growth of another species, i.e. buffalo calves.

MATERIALS AND METHODS

5.1.1 Plan of the experiment:

Twenty-four male buffalo calves of Murrah breed, weaned at birth were taken for this study. On 10th day, they were randomly distributed into four groups of six calves each. One group (1) was kept as control and the

other three groups (2, 3 and 4) were fed aflatoxin at the levels of 0.3, 0.6 and 1.0 ppm respectively on the basis of their dry matter intake. This study consisted of two phases: Phase 1: started from 10th day to 15 weeks of age and Phase II: started from 18 weeks to 30 weeks of age. All the buffalo calves were fed on milk in phase I, while in phase II, they were given concentrate mixture and green roughage. Fortnightly blood samples were collected for the estimation of immunoglobulin in phase I and weekly body weights were taken to assess their growth rate during both the phases of this study.

5.1.2 Selection and distribution of animals:

All the calves were not available at the same time, therefore, the experiment was staggered as per the calving and availability of buffalo calves on random basis. The particulars of buffalo calves and their distribution are presented in Table 14.

5.1.3 Housing and management:

All the buffalo calves were kept in well ventilated individual pens of size 1.5 m x 2.0 m. Each pen was provided with a raised iron platform having 2.5 cm size, wire net floor. The pens were thoroughly white-washed and disinfected before the start of the experiment. All the pens were disinfected daily in the morning at the time of cleaning and white-wahsed periodically. To avoid heat stress, cold water was sprinkled on buffalo calves twice daily. Tarpaulin was used to cover the front and sides of the calf pens at night hours. The buffalo calves were taken out for

Table 14. Particulars of male Murrah buffalo calves and their distribution

Treatment group	Sl. No.	Calf No.	Date of birth	Birth weight (kg)
Group 1 (0 ppm Af) Control	1	M 3157	7.8.86	36.0
	2	M 3158	9.8.86	39.0
	3	M 3161	15.8.86	34.0
	4	M 3166	21.8.86	23.0
	5	M 3186	3.10.86	34.0
	6	M 3206	18.11.86	35.0
	Mean			33.50
Group 2 (0.3 ppm Af)	1	M 3153	27.7.86	35.0
	2	M 3159	10.8.86	31.0
	3	M 3169	20.8.86	30.0
	4	M 3176	10.9.86	37.0
	5	M 3179	20.9.86	36.0
	6	M 3184	1.10.86	39.0
	Mean			34.67
Group 3 (0.6 ppm Af)	1	M 3154	2.8.86	33.0
	2	M 3165	20.8.86	31.0
	3	M 3172	29.8.86	27.0
	4	M 3178	17.9.86	39.0
	5	M 3189	8.10.86	32.0
	6	M 3237	19.1.87	37.0
	Mean			33.17
Group 4 (1.0 ppm Af)	1	M 3213	29.1..86	24.0
	2	M 3238	19.1.87	41.0
	3	M 3244	8.2.87	35.0
	4	M 3245	8.2.87	38.0
	5	M 3246	8.2.87	35.0
	6	M 3247	9.2.87	35.0
	Mean			34.67

exercise daily in the morning for half an hour. The calves were weaned at birth. After birth, each calf was cleaned well and the umbilical cord was divided leaving just 2.5 cm length from umbilicus. The umbilical area was washed with an antiseptic lotion and a swab of tincture was inserted. Birth weight of the buffalo calves was taken on a platform scale. The buffalo calves were ear numbered by tatooing on the first day. They were dehorned by electrical cauterization on the 6th day.

Package of practices as specified by Arora (1982) were followed as part of protective health cover. The details of the practices followed,are presented in Table 15. At the start of phase II, all the buffalo calves were dewormed with piperazine citrate 40% solution ICI at the rate of 5 ml per 10 kg body weight. Deworming was done at monthly intervals, alternately with piperazine citrate solution and panacur powder which contained 25 per cent Fenbendazole (Hoechst). Panacur was administered at the rate of 200 mg per 10 kg body weight as a suspension in water. The buffalo calves were given a wash with gammexane at the start of the experiment to remove infestation by ticks and lice, if any. They were also protected against Anthrax, Black Quarter, Haemorrhagic Septicaemia and Foot and Mouth disease by preventive inoculations.

5.1.4 Feeding of buffalo calves:

The buffalo calves were fed colostrum for first 5 days. The feeding of buffalo calves on colostrum and milk was based on their body weight according to the feeding

Table 15. Package of practices for buffalo calves

Age (days)	Health cover schedule	Dose	Preventive against
1	Orally auromycin	2 spoonful	Calf scour
2	Vitablend water miscible liquid (1,00,000 I.U. of vitamin A per ml)	1 ml	Night blindness
3 & 7	Piperazine adipate	2 spoonful	Ascariasis and dysentry
4	Sulphadiamidine 5 g tablet	½ tablet	Diarrhoea/ calf scour
8	Sulmet liquid (12.5% sodium sulphadimethyl pyridine)	30 ml	Coccidiosis
9,10,11	-do-	20 ml	Coccidiosis

schedule given in Table 16 as described by Arora (1982). Milk was fed daily in two equal portions, in the morning at 9.00 A.M. and in the evening at 4.00 P.M. Before feeding, milk was boiled and cooled to room temperature. Feeding was done in plastic pails with fingers dipped in the fluid to enable suckling by the calves. Mineral mixture (Table 10) was also given daily to all the calves as per their requirement. Rovimix in oil (10,000 I.U. of vitamin A) was also administered once a week to all the calves. This feeding practice continued throughout the first phase of the experiment.

After the completion of phase I, buffalo calves were gradually shifted from milk to concentrate and green fodder feeding. During this change over of 3 weeks, they were not given aflatoxin. During the post-ruminant age, the calves were fed on concentrate mixture and green maize/ oat as per their requirements (NRC, 1978). The details of feeding schedule are given in Table 17. The concentrate mixture consisted of crushed maize: 40 parts; groundnut cake: 32 parts; wheat bran: 25 parts and mineral mixture: 3 parts. The concentrate mixture contained 20.5% CP and 70.65% TDN on dry matter basis (Table 13). Weighed quantity of concentrate mixture and green fodder was offered at about 9.00 A.M. and 12.30 P.M. respectively. Clean drinking water was provided liberally to all the buffalo calves twice daily.

5.1.5 Recording of weight gain and dry matter feed intake:

Weekly weight gain and daily dry matter intake were recorded as described in Chapter 4.1.4

Table 16. Feeding schedule (pre-ruminant)

Body weight (kg)	Colostrum/milk (kg)
Upto 25	1.0
26 - 35	2.0
36 - 40	2.5
41 - 45	3.0
46 - 50	3.5
51 - 55	4.0
56 - 60	4.5
61 and above	5.0

Table 17. Feeding schedule (post-ruminant)

Body weight (kg)	Concentrate		Green fodder		Total		
	FM (kg)	DM (kg)	FM (kg)	DM (kg)	DM (kg)	TDN (kg)	CP (g)
50	0.8	0.7	4.0	0.8	1.5	0.98	207
60	1.0	0.9	4.5	0.9	1.8	1.18	257
70	1.2	1.1	5.0	1.0	2.1	1.39	307
80	1.4	1.2	6.0	1.2	2.4	1.58	342
90	1.5	1.3	7.0	1.4	2.7	1.77	378
100	1.6	1.4	8.0	1.6	3.0	1.96	413
110	1.7	1.5	8.5	1.7	3.2	2.09	442
120	1.8	1.6	9.0	1.8	3.4	2.22	470

Table 18. Composition of concentrate mixture

Ingredients	Parts	CP	TDN
Maize	40	4.00	32.00
Groundnut cake	32	13.40	22.40
Wheat bran	25	3.10	16.25
Mineral mixture	3		
Total	100	20.50	70.65

5.1.6 Collection of blood samples:

Ten ml of samples of blood were collected under sterile conditions from the jugular puncture. 4 cm long stainless steel hypodermic needles of 18 gauge and 30 ml glass test tubes were used for the collection of blood samples. The initial blood samples were collected on 10th day, prior to aflatoxin feeding and subsequent blood samples were collected at an interval of 15 days throughout phase I.

5.1.7 Determination of total immunoglobulins in blood serum

Immunoglobulin levels in the blood serum were determined by zinc sulphate turbidity test (McEwan et al., 1970) with standard curve prepared from standard bovine Ig Cohn fraction II, obtained from Sigma Chemical Co., U.S.A.

5.1.8 Production of crude aflatoxin mixture:

Crude aflatoxin mixture was produced in the laboratory by growing Aspergillus parasiticus NRRL 3240 on a synthetic low salt medium (Reddy et al., 1971) containing sucrose, asparagine and various mineral salts (Table 19) in Haffkine's culture flasks. The culture flasks were incubated in a B.O.D. incubator at 26°C ± 1°C for 8 days as stationary cultures. Toxin was released into the nutrient medium as a result of extensive mycelial growth.

Nutrient medium from the culture flasks was collected in a 5 litre conical flask. It was extracted with 1.5 litres of chloroform and re-extracted thrice with 500 ml chloroform each time. The chloroform extracts were pooled

Table 19. Composition of synthetic low-salt medium used for aflatoxin production

Ingredients	Amount (g)
Sucrose	85.000
Asparagine	10.000
$(NH_4)_2SO_4$	3.500
KH_2PO_4	0.750
$MgSO_4.7H_2O$	0.350
$CaCl_2.2H_2O$	0.075
$ZnSO_4.7H_2O$	0.010
$MnCl_2.4H_2O$	0.005
$(NH_4)_6Mo_{24}$ $4H_2O$	0.002
$Na_2B_4O_7.10H_2O$	0.002
$FeSO_4.7H_2O$	0.002
Distilled water to make a volume of 1,000 ml (pH adjusted to 4.5 using 0.1 N HCl before making up the final volume)	

and condensed to a volume of 50 ml by evaporating in a rotary evaporator under vacuum. The condensed chloroform extract was taken in a 1 litre beaker and added with 500 ml hexane, which resulted in the precipitation of the crude aflatoxin. The beaker was kept at 4°C in a refrigerator for 12 hours to enable complete precipitation. Crude aflatoxin was later separated by filtering through Whatman filter paper No.1. It was air-dried, weighed and stored in a clean glass vial, covered with aluminium foil, at 4°C in a refrigerator. The crude aflatoxin mixture was quantified by thin layer, chromatographic separation and fluorimetric determination (A.O.A.C., 1980; Davis et al., 1980) or on High Pressure Liquid Chromatography (Water, Model No.510) by using aflatoxin standards for B_1, B_2, G_1 and G_2 procured from Sigma Chemical Co., St. Louis, U.S.A.

5.1.9 Administration of aflatoxin:

Fine maize flour was used as a carrier for administration of aflatoxin. Crude aflatoxin mixture (B_1 = 42% and G_1 = 27%) was thoroughly and uniformly mixed with maize flour in appropriate amount so that the final mixture contained 1 mg of pure total aflatoxin per gram. Required amount of this mixture was weighed and taken on a cellulose paper as per the individual dose level of each calf. The cellulose paper containing the mixture was folded into a packet and fed directly into the mouth of the calf, prior to morning feeding. Proper care was taken to avoid any spilling while administering the toxin. Weekly adjustments

were made in aflatoxin doses according to weekly changes of dry matter intake.

5.1.10 Statistical analysis of the data:

The data were analysed according to Snedecor and Cochran (1967). Missing plot technique in random block design was used to analyse the growth data in phase II because one calf in group 4 died due to diarrhoea during the change over period.

RESULTS AND DISCUSSION

5.2.1 Immunoglobulins concentration in blood serum:

Occasional screening of milk samples fed to buffalo calves revealed that milk samples initially did not contain any aflatoxin M_1 . Hence, all the aflatoxin received by the buffalo calves in the various treatments were only by way of supplementation of crude aflatoxin mixture at the stipulated level of 0, 0.3, 0.6 and 1.0 ppm.

The blood serum Ig levels of buffalo calves of 4 treatment groups during the course of study in phase I are presented in Table 20. The initial value of Ig (mg/ml blood serum) on day 10 was 25.39 ± 6.90, 28.59 ± 6.76, 23.78 ± 6.34 and 24.82 ± 1.98 respectively in 4 groups. Statistically these values were not different from each other, but there was a significant decrease ($P < 0.01$) in Ig level with incremental increase in aflatoxin level(Fig.7). The decline in Ig concentration in blood serum of buffalo calves given aflatoxin at 1.0 ppm level, started from 40 days of their age, while in buffalo calves given aflatoxin

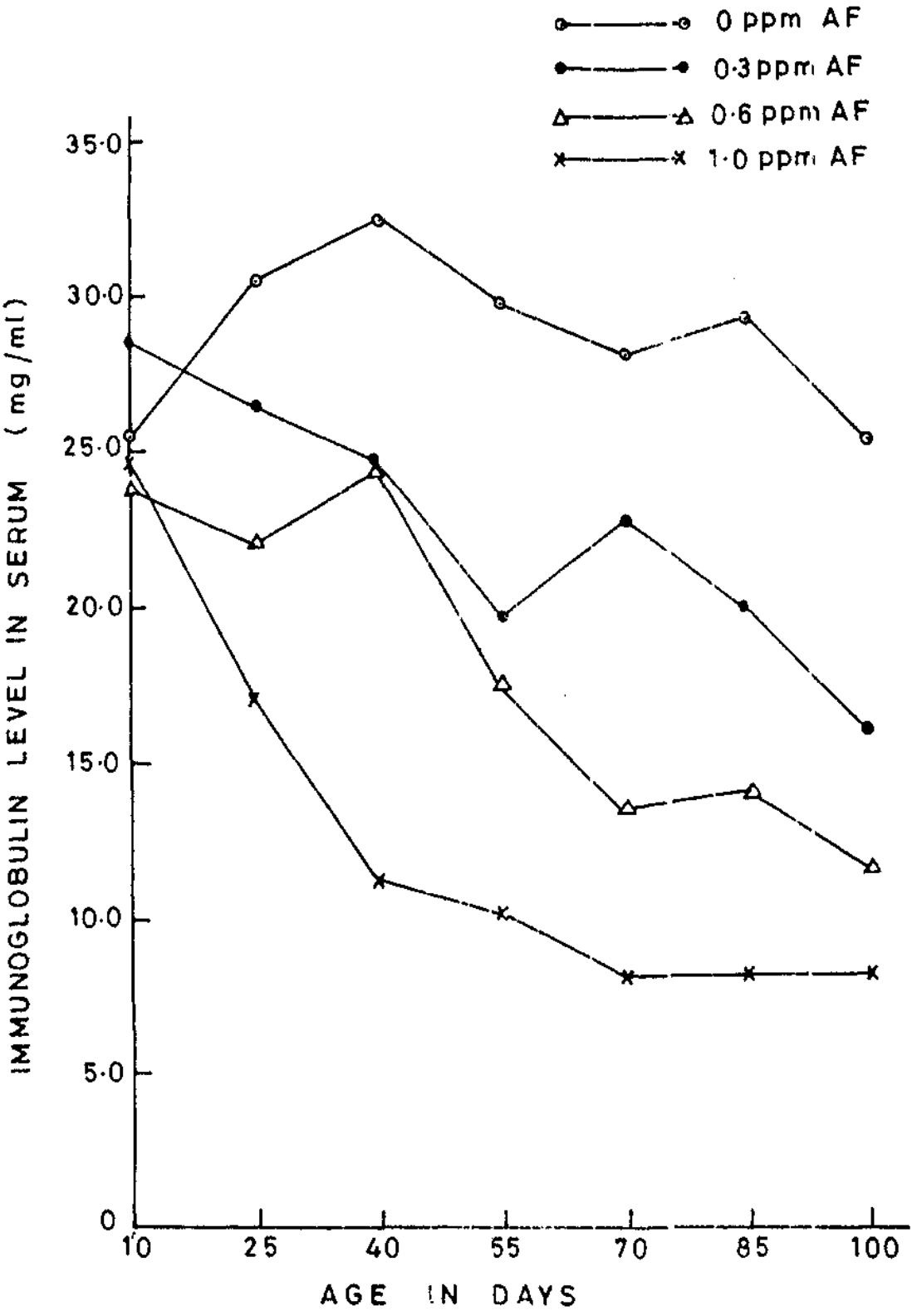
Table 20. Effect of feeding different levels of aflatoxin on Ig level in blood serum (mg/ml)

Age (days)	GROUP			
	1(0 ppm Af)	2 (0.3 ppm Af)	3 (0.6 ppm Af)	4 (1.0 ppm Af)
10	25.39 ± 6.90	28.59 ± 6.76	23.78 ± 6.34	24.82 ± 1.98
25	30.49 ± 4.90	26.39 ± 5.59	22.12 ± 5.33	17.09 ± 1.47
40**	32.40^{a} ± 6.17	24.83^{a} ± 2.67	24.45^{a} ± 4.17	11.23^{b} ± 1.57
55**	29.75^{a} ± 5.36	19.67^{ac} ± 2.45	16.51^{bc} ± 2.62	10.31^{b} ± 1.04
70*	28.11^{a} ± 6.59	22.87^{ac} ± 2.25	13.35^{bc} ± 2.13	8.13^{b} ± 1.78
85**	29.22^{a} ± 6.57	20.14^{ab} ± 4.14	14.13^{bc} ± 1.33	8.10^{c} ± 0.93
100**	25.30^{a} ± 2.07	16.13^{b} ± 2.96	11.65^{bd} ± 1.60	8.19^{cd} ± 0.87

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

Values bearing same superscript in the same row do not differ significantly from each other

FIG. 7. IMMUNOGLOBULIN LEVELS IN SERUM OF BUFFALO CALVES FED DIFFERENT LEVELS OF AFLATOXIN (PHASE I)



at 0.6 and 0.3 ppm levels it started declining at the age of 55 and 100 days respectively. Arora et al.(1988b)reported a decrease in Ig concentration (mg/ml) in blcod serum of kids fed graded levels of aflatoxin. Balaraman and Arora (1987) also observed a significant decrease in Ig concentration in blood serum of neonatal crossbred calves as a result of aflatoxin feeding.

Ig absorption in the bovine neonate is reported to take place by pinocytotic activity of the intestinal epethelial cells and the transport to blood is by way of lymphatics (Comline et al.,1951). The Ig-ferritin conjugate formed within the absorptive cell is transported by the apical tubular system and released at the basal cell membrane for absorption (Staley et al., 1972). The declining trend of Ig level in blood serum of buffalo calves in the present study may be due to interference of aflatoxins in Ig-ferritin conjugate formation and hence its less transport to the apical tubular system.

No intrinsic synthesis of Ig would occur until the calves were 4 weeks of age (Logan et al., 1974). Calf serum would remain free of intrinsic Ig for several weeks, acquiring typical pattern only after 6 weeks of age (Larson et al., 1980). In the present study, the Ig concentration (mg/ml blood serum) was 32.40 ± 6.17 at 40 days of age in control group, which remained almost constant upto the end of this study, i.e. 100 days of age. But in aflatoxin treatment groups, there was a progressive decline in Ig concentration (mg/ml blood serum) at all levels of aflatoxin.

Decline was more pronounced at higher levels of aflatoxin as compared to low level. The reason of low Ig value in these groups may be due to the adverse effect of aflatoxin on intrinsic synthesis of Ig.

The result of the present study indicated that dietary aflatoxin at 0.3, 0.6 and 1.0 ppm levels significantly decreased the blood serum Ig level (mg/ml) and thus affected the passive immunity of young buffalo calves. Interference in the intrinsic synthesis could be the principal reason for their lowered immune status.

5.2.2 Growth rate and feed efficiency (Phase I):

Initial body weights of buffalo calves in different groups were: Group I: 35.83 ± 2.23, Group 2: 36.00 ± 1.23; Group 3: 34.50 ± 1.92 and Group 4: 36.33 ± 2.12. They did not vary significantly amongst themselves. Details of weekly weight gains (kg) in different groups are given in Table 21. The average weekly weight gains (kg) were: Group 1: 2.82 ± 0.10; Group 2: 2.23 ± 0.13; Group 3: 1.85 ± 0.04 and Group 4: 1.44 ± 0.14 indicating the decline in weight gain with the increase in aflatoxin level. The cumulative weekly weight gains (kg) in different groups are given in Table 22 and depicted in Fig. 8 The total weight gains (kg) during 13 weeks period were: Group 1: 36.61 ± 2.11, Group 2: 29.33 ± 1.17, Group 3: 24.00 ± 2.02 and Group 4: 18.67 ± 1.28. The average daily gains (g) in different groups were: Group 1: 402.3 ± 12.8; Group 2: 318.5 ± 12.4; Group 3: 264.3 ± 9.2 and Group 4: 205.7 ± 13.8. Regression analysis of cumulative weight gains of

Table 21. Weekly weight gains (kg) in different groups (phase I)

Weeks	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
1	2.25±0.42	2.58±0.24	2.25±0.17	2.08±0.27
2	2.42±0.44	1.75±0.21	1.92±0.08	1.58±0.20
3	2.75±0.41	**1.89±0.20	**2.20±0.17	**1.83±0.11
4	2.67±0.21	**2.08±0.27	2.25±0.17	*1.58±0.20
5	2.92±0.27	2.42±0.20	*2.25±0.16	*1.83±0.17
6	2.75±0.17	2.25±0.21	**1.92±0.25	**1.50±0.22
7	3.09±0.30	**1.92±0.30	**1.75±0.26	**1.25±0.17
8	2.92±0.20	**2.24±0.21	**1.83±0.28	**0.92±0.20
9	2.92±0.27	**1.84±0,28	**1.58±0.46	**1.17±0.17
10	2.92±0.33	2.67±0.33	**1.67±0.33	**1.0±0.22
11	3.00±0.29	**2.33±0.27	**1.75±0.36	**1.33±0.25
12	2.92±0.53	2.67±0.21	**1.75±0.42	**1.17±0.11
13	3.08±0.46	2.66±0.22	**1.67±0.44	**1.25±0.31

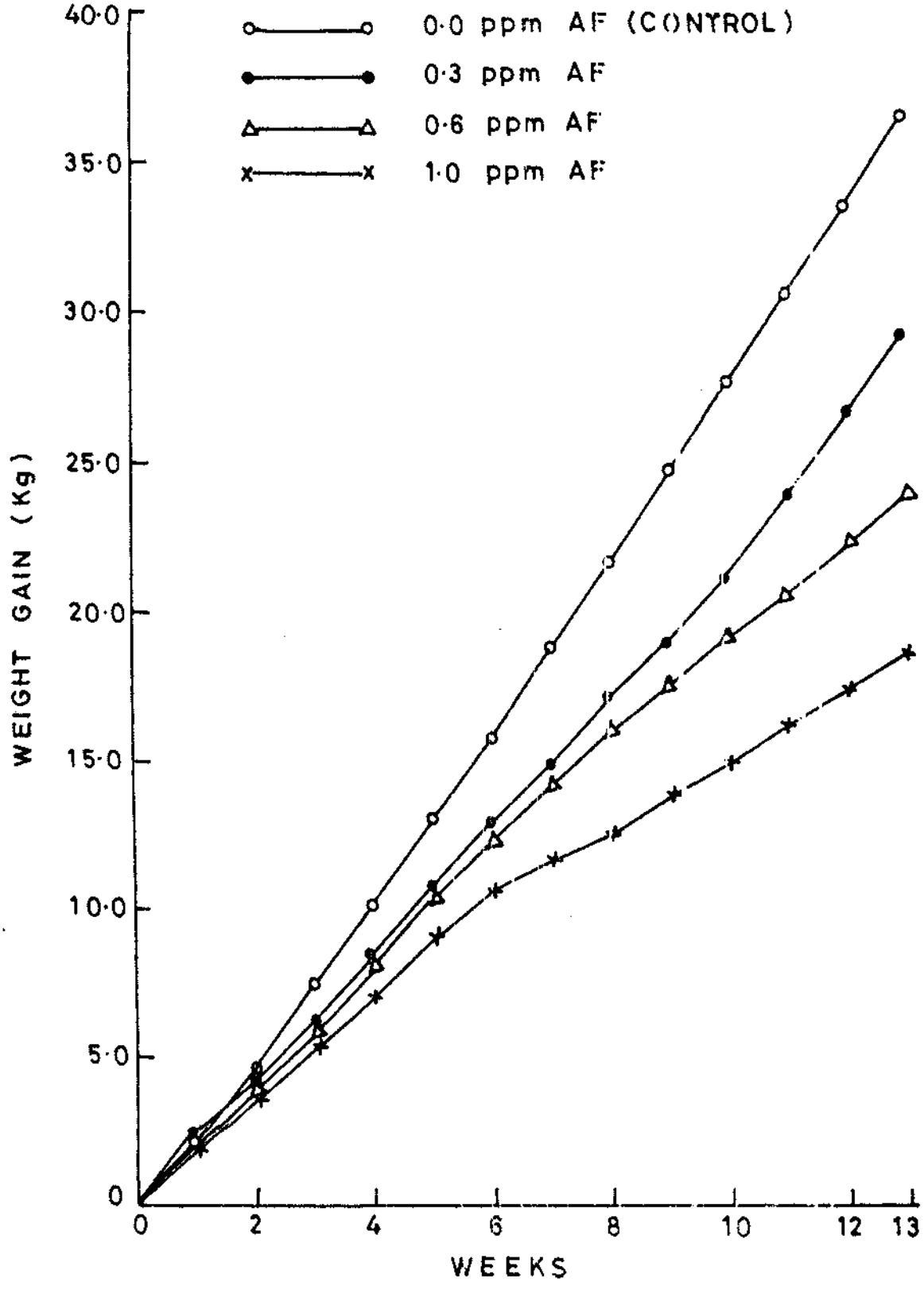
* $P < 0.05$; ** $P < 0.01$

Table 22. Cumulative weekly weight gains (kg) in different groups (phase I)

Weeks	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
1	2.25±0.42	2.58±0.24	2.25±0.17	2.08±0.27
2	4.67±0.31	4.33±0.40	4.17±0.17	3.58±0.38
3	7.42±0.46	**6.22±0.44	**6.0±0.00	**5.40±0.33
4	10.09±0.64	**8.33±0.52	**8.25±0.17	**7.08±0.27
5	13.01±0.79	**10.75±0.62	**10.50±0.22	**9.00±0.37
6	15.76±0.84	**13.00±0.62	**12.42±0.46	**10.50±0.34
7	18.86±0.82	**14.92±0.86	**14.17±0.60	**11.75±0.44
8	21.77±0.98	**17.16±0.92	**16.00±0.81	**12.67±0.56
9	24.69±1.19	**19.00±0.97	**17.50±1.10	**13.83±0.65
10	27.61±1.49	**21.33±0.92	**19.17±1.04	**14.92±0.88
11	30.61±1.62	**24.00±1.03	**20.58±1.38	**16.25±1.08
12	33.53±1.70	**26.67±1.09	**22.33±1.73	**17.42±1.16
13	36.61±2.11	**29.33±1.17	**24.00±2.02	**18.67±1.28

** $P < 0.01$

FIG. 8. CUMULATIVE WEEKLY WEIGHT GAINS OF BUFFALO CALVES IN DIFFERENT GROUPS (PHASE I)



buffalo calves during 13 weeks gave the 'b' value (kg) per week as follows: Group 1: 2.76 ± 0.19; Group 2: 2.15 ± 0.12; Group 3: 1.86 ± 0.18 and Group 4: 1.50 ± 0.07, indicating a progressive reduction in weekly weight gain with incremental increase in the level of aflatoxin (Table 23).

Statistical analysis of the data revealed that weekly weight gains were significantly reduced ($P < 0.01$) from 3rd week onwards in all the aflatoxin fed groups. The data on weight gain thus showed that all the aflatoxin levels tested, namely, 0.3, 0.6 and 1.0 ppm adversely affected the growth rate.

Details of dry matter intake and feed:gain ratios in different treatment groups are given in Table 24. Total dry matter consumption (kg) and average daily dry matter consumption (kg) in different treatment groups were: Group 1: 49.06 ± 2.44 and 0.540 ± 0.027; Group 2: 48.07 ± 2.07 and 0.529 ± 0.022; Group 3: 46.61 ± 1.4 and 0.513 ± 0.015 and Group 4: 46.28±2.71 and 0.509±0.030. Statistical analysis of the data did not reveal any difference in these parameters. Feed to gain ratios were 1.34 ± 0.058, 1.65 ± 0.056, 2.14 ± 0.176 and 2.69 ± 0.184 in groups 1, 2, 3 and 4 respectively, indicating higher dry matter consumption per unit weight gain with increase in aflatoxin levels.

In the present study effect of aflatoxin on growth rate was taken as the basis of assessing the safe level for buffalo calves and thus a regression line was drawn (Fig. 9). For this, growth rate in different groups were plotted against graded levels of aflatoxin. On the basis

FIG. 9. REGRESSION LINE FOR AVERAGE DAILY GAINS IN DIFFERENT GROUPS OF BUFFALO CALVES (PHASE I)

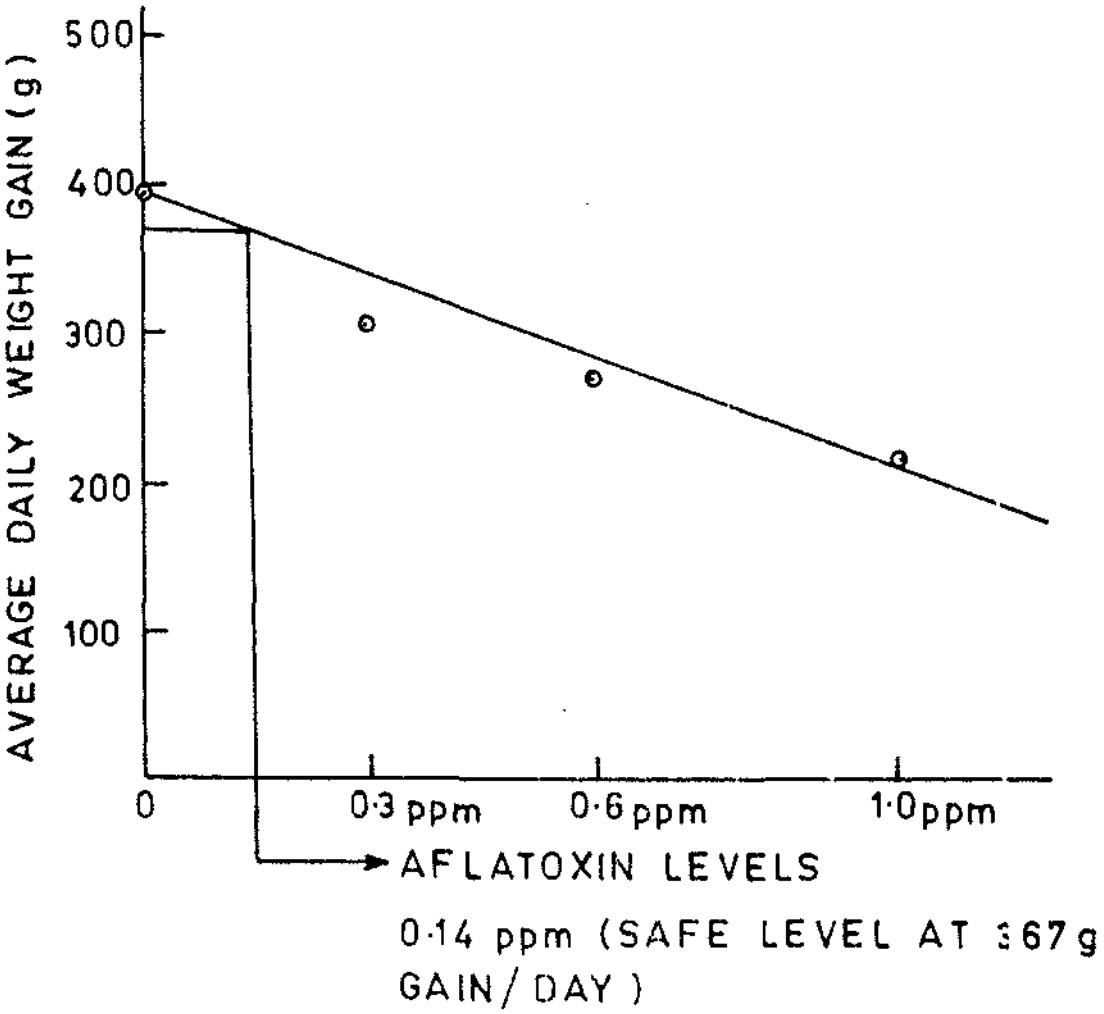


Table 23. Effect of graded levels of aflatoxin on growth rate (phase I)

Attributes	GROUP			
	1(0 ppm Af)	2(0.3 ppm Af)	3(0.6 ppm Af)	4(1.0 ppm Af)
Initial weight (kg)	35.83±2.23	36.0±1.23	34.50±1.92	36.33±2.12
Final weight (kg)	72.44±3.96	65.33±0.75	58.50±3.49	55.00±2.70
Weight gain (kg)	36.61±2.11	**29.33±1.17	**24.00±2.02	**18.67±1.28
Weight gain/ week (kg)	2.82±0.10	**2.23±0.13	**1.85±0.04	**1.44±0.14
Weight gain/day (g)	402.30±12.80	**318.5±12.40	**264.3±9.20	**205.70±13.80
Regression (b) value/week (kg)	2.76±0.19	**2.15±0.12	**1.86±0.18	**1.50±0.07

** $P < 0.01$

Table 24. Dry matter consumption and feed:gain ratio (phase I)

Attribute	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
Total DM consumption (kg)	49.06±2.44	48.07±2.07	46.61±1.40	46.28±2.71
Average daily DM consumption (kg)	0.540±0.027	0.529±0.022	0.513±0.015	0.509±0.030
Feed:gain ratio	1.34±0.058	1.65±0.056	**2.14±0.176	**2.69±0.184

** $P < 0.01$

of this regression line, it was inferred that 0.14 ppm might be the safe level for growth in buffalo calves (pre-ruminant).

5.2.3 Growth rate and feed conversion efficiency (Phase II):

During the second week of change over, one calf No. 3247 died due to diarrhoea. So, the data in this group was based on 5 buffalo calves only.

The initial body weights of buffalo calves in different treatment groups were: 75.17 ± 3.82, 69.41 ± 2.23, 62.25 ± 4.10 and 57.1 ± 2.87 in four groups respectively. Details of weekly weight gains (kg) in different treatment groups are given in Table 25. The average weekly weight gains were: Group 1: 2.80 ± 0.18; Group 2: 2.06 ± 0.15; Group 3: 1.58 ± 0.19; and Group 4: 1.41 ± 0.07, indicating the decline in weight gains with the increase in aflatoxin level. The cumulative weekly weight gains (kg) in different groups are given in Table 26 and depicted in Fig. 10. The total weight gains (kg) during 12 weeks period of growth were 33.63 ± 0.90; 24.75 ± 1.79; 18.92 ± 2.32 and 16.90 ± 0.81 respectively. The average daily gain (kg) in different groups were: Group 1: 0.400 ± 0.02; Group 2: 0.295 ± 0.02; Group 3: 0.225 ± 0.03 and Group 4: 0.202 ± 0.09. Regression analysis of cumulative weight gains during 12 weeks gave the 'b' value (kg) per week as follows: Group 1: 2.81 ± 0.250; Group 2: 2.03 ± 0.180; Group 3: 1.50 ± 0.024 and Group 4: 1.42 ± 0.090 indicating an adverse effect of aflatoxin on growth (Table 27). Analysis of variance of the data revealed that weekly weight gains were reduced from 4th week onwards

Table 25. Weekly weight gains (kg) in different groups (Phase II)

Weeks	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
1	2.50±0.22	2.08±0.20	2.08±0.30	1.50±0.20
2	2.83±0.17	2.15±0.34	2.25±0.40	1.00±0.32
3	2.67±0.21	2.12±0.40	2.00±0.50	1.50±0.32
4	2.92±0.20	2.32±0.20	*1.92±0.33	*1.90±0.25
5	2.75±0.25	2.42±0.20	1.75±0.25	1.70±0.44
6	2.25±0.51	2.42±0.37	*1.58±0.42	*1.40±0.17
7	2.67±0.25	1.92±0.24	**1.50±0.22	**0.70±0.49
8	2.75±0.17	2.08±0.27	**1.00±0.68	**1.20±0.20
9	2.83±0.17	*1.50±0.76	*1.75±0.57	*1.60±0.25
10	3.00±0.00	**1.42±0.89	**1.58±0.52	**1.30±0.37
11	2.92±0.20	**2.17±0.31	**0.50±0.34	**1.60±0.29
12	3.08±0.08	**2.17±0.31	**1.50±0.34	**1.70±0.31

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

Table 26. Cumulative weight gains (post-ruminant)

Week	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
1	2.50±0.22	2.08±0.20	2.08±0.30	1.50±0.22
2	5.33±0.21	4.33±0.37	4.33±0.64	2.50±0.22
3	8.00±0.37	6.41±0.51	6.33±1.08	4.00±0.38
4	10.92±0.52	8.67±0.54	** 8.25±1.09	** 5.70±0.57
5	13.67±0.67	10.92±0.55	**10.00±1.24	** 7.40±0.81
6	16.42±0.88	13.33±0.85	**11.58±1.59	** 9.00±0.79
7	19.08±1.04	15.25±0.99	**13.08±1.79	** 9.70±0.92
8	21.83±0.95	17.33±0.99	**14.08±2.11	**10.90±0.90
9	24.67±1.05	18.33±1.16	**15.50±2.39	**12.30±0.89
10	27.67±1.06	**20.42±1.56	**17.08±2.45	**13.60±1.04
11	30.58±1.04	**22.25±1.99	**17.58±2.48	**15.20±1.10
12	33.67±1.08	**24.42±1.99	**18.92±2.54	**16.90±0.90

** $P < 0.01$

FIG. 10. CUMULATIVE WEEKLY WEIGHT GAINS OF BUFFALO CALVES IN DIFFERENT GROUPS (PHASE II)

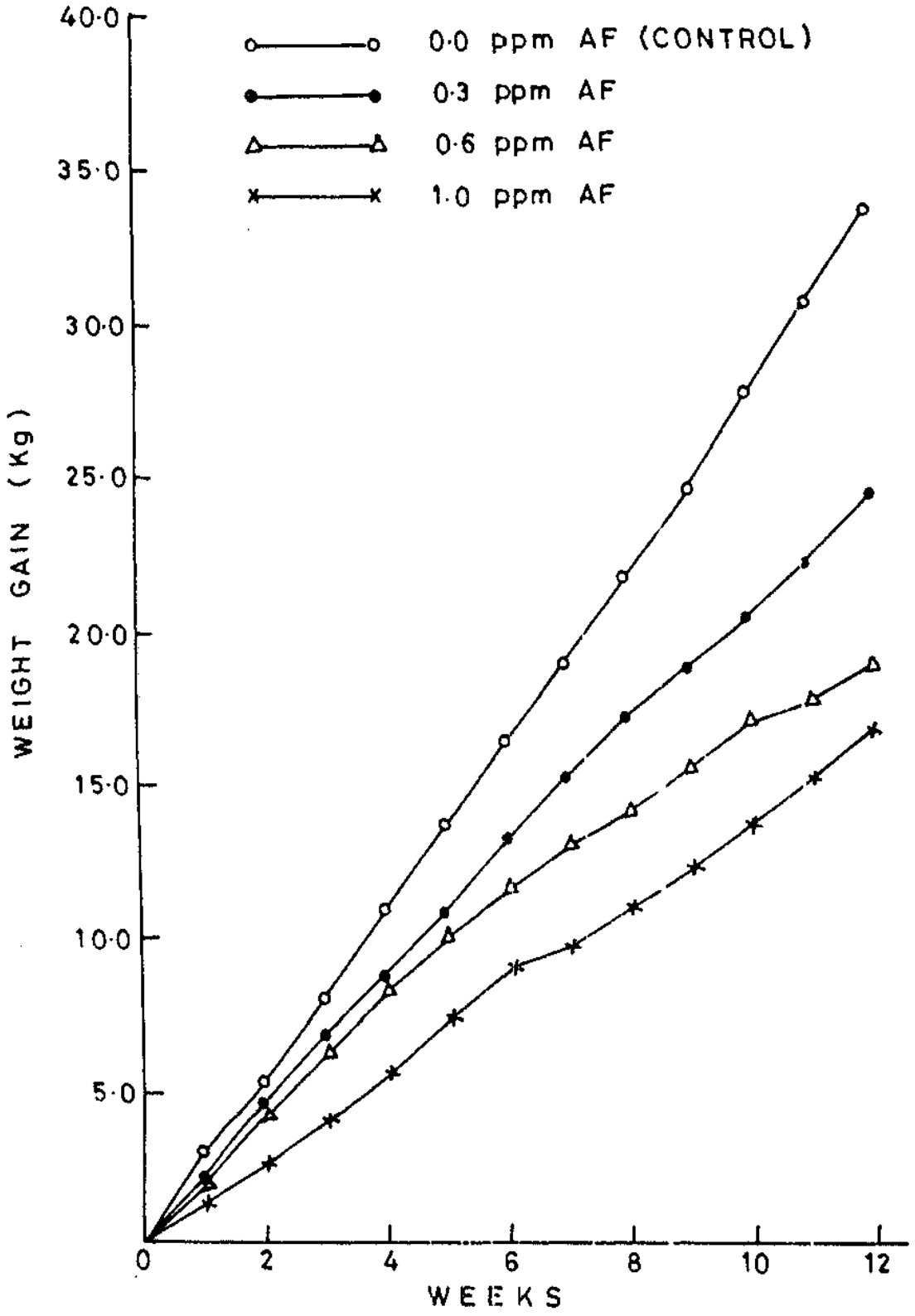


Table 27. Effect of graded levels of aflatoxin on growth rate (phase II)

Attribute	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
Initial weight (kg)	75.17±3.82	69.41±2.23	62.25±4.10	57.10±2.87
Final weight (kg)	108.80±4.50	94.17±3.05	81.17±6.01	74.00±3.18
Weight gain (kg)	33.63±0.90	24.75±1.79	**18.92±2.32	**16.90±0.81
Weight gain/week (kg)	2.80±0.10	2.06±0.15	** 1.58±0.19	** 1.41±0.07
Weight gain/day (kg)	0.400±0.02	0.295±0.02	**0.225±0.03	**0.202±0.09
Regression (b) value/week (kg)	2.81±0.25	2.03 ±0.18	**1.50 ±0.024	**1.42 ±0.098

** $P < 0.01$

in groups 3 and 4 and from 9th week onwards in group 2.

The details of dry matter intake and feed:gain ratios in different groups are given in Table 28. Total dry matter consumption (kg) and average daily dry matter consumption (kg) in different treatment groups were: Group 1: 206.83 ± 11.14 and 2.46 ± 0.133; Group 2: 206.98 ± 2.99 and 2.50 ± 0.127; Group 3: 200.58 ± 3.42 and 2.39 ± 1.60; and Group 4: 202.5 ± 1.40 and 2.41 ± 0.15. There was no significant difference in these parameters. Corresponding feed:gain ratios were: 6.15 ± 0.28; 8.62 ± 0.65; 11.41 ± 1.28 and 12.1 ± 0.52, indicating higher dry matter consumption per unit weight gain with increase in aflatoxin levels($P < 0.01$).

A regression line (Fig. 11) was also drawn to determine the safe level of aflatoxin for post-ruminant buffalo calves as described in phase I. On the basis of this regression line, it was inferred that 0.15 ppm might be the safe level for growth in buffalo calves (post-ruminant).

Results of the effect of feeding graded levels of aflatoxin during phase I and phase II indicated an adverse effect on growth rate. The effects were more pronounced at higher levels (0.6 and 1.0 ppm) than at lower level (0.3 ppm). Similar results have been reported in other species by some workers (Allcroft and Lewis, 1963 a,b; Horrocks et al., 1965; Flatla et al., 1969; Lynch et al. 1971; Neathery et al., 1980; Patterson and Anderson, 1982; Balaraman and Arora, 1986).

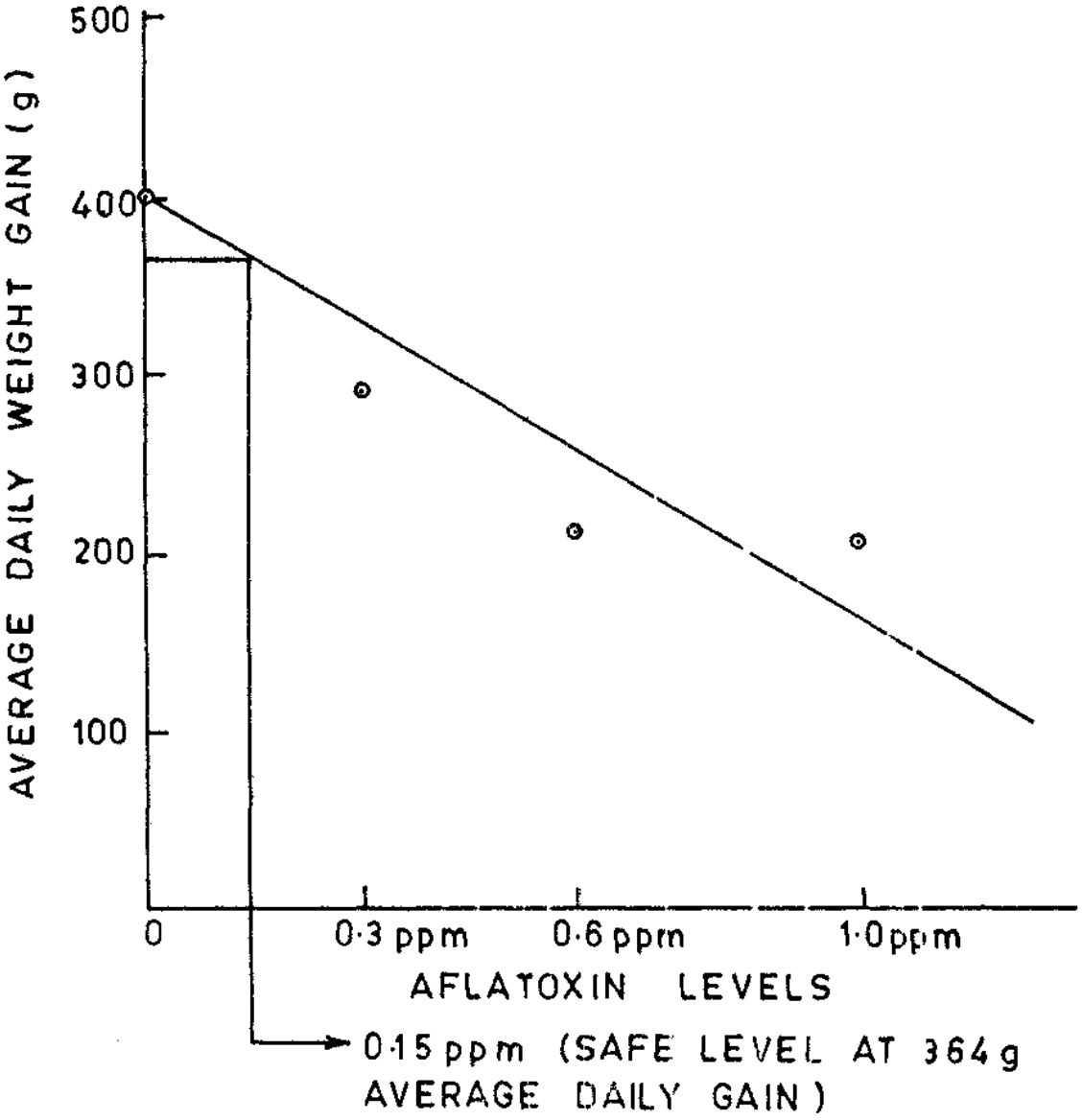
Allcroft and Lewis (1963b) observed the retardation

Table 28. DM consumption and feed to gain ratios (phase II)

Attribute	Group 1 (0 ppm Af) Control	Group 2 (0.3 ppm Af)	Group 3 (0.6 ppm Af)	Group 4 (1.0 ppm Af)
Total DM consumption (kg)	206.83±11.14	206.98±2.99	200.58±3.42	202.50±1.40
Average daily DM consumption (kg)	2.46±1.33	2.50±1.27	2.39±1.60	2.41±0.15
Feed:gain ratio	6.15±0.28	8.62±0.65	**11.41±1.28	**12.10±0.52

** P< 0.01

FIG.11. REGRESSION LINE FOR AVERAGE DAILY GAINS IN DIFFERENT GROUPS OF BUFFALO CALVES (PHASE II)



of growth in calves fed a diet containing toxic groundnut meal. Horrocks et al. (1965) reported a decrease in growth rate in Ayrshire bull calves fed 0.22 to 0.44 ppm aflatoxin. Flatla et al. (1969) found a depression in weight gain in bull calves given 204 to 474 ppb aflatoxin for 4 months. Lynch et al. (1971) studied the effect of different levels of aflatoxin ranging from 0 to 0.10 mg/kg body weight in calves. There was a significant reduction ($P < 0.01$) in weight gain with the increase in dose and period of treatment. Neathery et al. (1980) fed young Holstein bull calves a diet containing 3 ppm aflatoxin and observed significant reduction in feed intake, average daily gain and nitrogen retention. Balaraman and Arora(1936) reported a significant decrease in weight gain and feed conversion efficiency in calves fed a diet containing graded levels of aflatoxin varying from 0 to 1.5 ppm.

Contrary to above reports, Garret et al. (1968) did not observe any adverse effect on the growth of calves provided upto 0.3 ppm level of aflatoxin. Keyl and Booth (1971) also observed similar results in young beef cattle fed aflatoxin. Similarly, Eddy et al. (1980a) could not find any adverse effect on the growth of Holstein heifers due to aflatoxin, fed upto 4.8 mg (.35 ppm) for 140 days. Keyl and Booth (1971) suggested that aflatoxin feeding at a level of 0.3 ppm in the diet was safe for calves. Patterson and Anderson (1982) agreed with the findings of Keyl and Booth (1971) and reported aflatoxin toxicity in calves appearing only when the diet was having aflatoxin more than 30 µg/kg feed.

In the present study, the dietary safe levels for pre-ruminant and post-ruminant buffalo calves were found to be 0.14 and 0.15 ppm respectively. These dietary safe levels of aflatoxins for buffalo calves are similar to pre-ruminant kids (Arora et al., 1988b). But the levels are much less as compared to safe value of 0.26 ppm in crossbred calves (Balaraman and Arora, 1986). This might be due to the reason that buffalo calves are more susceptible to aflatoxin toxicity than calves (Murthy et al., 1984).

There was no effect of feeding aflatoxin on total dry matter consumption in buffalo calves. This indicated that the decline in growth rate was not due to less feed intake in aflatoxin fed groups, rather impaired utilization of nutrients at tissue level by aflatoxin might be the cause of reduced growth and hence higher feed to gain ratios in these groups.

EFFECT OF AFLATOXIN ON NITROGEN DYNAMICS IN CATTLE AND BUFFALO CALVES FED DIFFERENT LEVELS OF DIETARY PROTEIN

Aflatoxin has been reported to induce wide variety of adverse effects in different species of livestock (Allcroft and Lewis, 1963; Armbrecht et al., 1970; Southern and Clawson, 1979; Murthy et al., 1984; Arora et al., 1988a), particularly it affects the nitrogen metabolism (Lynch et al., 1973; Hale and Wilson, 1979). It has also been reported that higher levels of protein in the diet have protective effect against aflatoxin toxicity (Edds, 1973; Hatch, 1977; Balaraman and Arora, 1984). The objective of this experiment was to have a comparative study on the effect of aflatoxin on nitrogen dynamics in calves fed optimum high and low levels of protein in the diet.

6.1 MATERIALS AND METHODS

6.1.1 Plan of the experiment:

This experiment was conducted on 8 male rumen fistulated, i.e., 4 cattle and 4 buffalo calves of 2-2½ years of age, completed in two phases. One crossbred calf each was kept in groups 1, 2, 5 and 6 while one buffalo calf each was allotted to groups 3, 4, 7 and 8. All the animals were fed on concentrate mixture, green fodder and wheat straw to meet their nutrient requirements (NRC, 1978). Animals in groups 1, 2, 3 and 4 were given 42.50 per cent more protein (HP) in phase I. In phase II, the animals were provided 51.47 per cent less protein (LP) in groups 5, 6, 7 and 8

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than optimum. In addition, animals in groups 2, 4, 6 and 8 were given an oral dose of aflatoxin @ 1.0 ppm of their dry matter intake. During phase I, animals on this dietary regimen were adapted for 40 days and a metabolism trial was conducted to know the digestibility of nutrients. After the metabolism trial, a single dose of ^{51}Cr-EDTA and ^{15}N ammonium sulphate was infused into the rumen of each animal and samples of rumen liquor, blood and urine were collected at various time intervals to know the rumen volume, digesta flow rate and nitrogen dynamics of these animals. Similarly, a metabolism trial and isotopic studies were conducted during phase II, after a preliminary feeding of 40 days.

6.1.2 Animals and feeding regimen

The distribution of animals and particulars of the treatments in phase I and phase II are presented in Table 29. Parts of different feed ingredients used for the formulation of concentrate mixtures are given in Table 30 and the chemical composition of feed ingredient offered to animals are given in Tables 31 and 32. The feeding schedule accpted during the experimental period is given in Table 33.

6.1.3 Housing of the animals:

The animals were kept in a well ventilated metabolic stall during the metabolism trial and later shifted to iron slotted cots for using radioisotopes to avoid any recontamination. The floor was cleaned daily with phenyl.

6.1.4 Digestibility studies:

The animals of both the species were fed on their

Table 29. Details of experimental calves in different groups

Species	Animal No.	Body weight (kg)	Group	Level of protein		Aflatoxin (ppm)
				Phase I	Phase II	
Cattle	KS-3443	325.0	1	High	Optimum	-
-do-	KS-4470	387.5	2	-do-	-do-	1.0
Buffalo	M-2680	369.0	3	-do-	-do-	-
-do-	M-2787	337.0	4	-do-	-do-	1.0
Cattle	KS-3450	335.0	5	Optimum	Low	-
-do-	KS-3442	360.0	6	-do-	-do-	1.0
Buffalo	M 2686	372.0	7	-do-	-do-	-
-do-	M-2714	370.0	8	-do-	-do-	1.0

Table 30. Percent composition of concentrate mixtures fed in Phases I and II

Ingredients	Phase I		Phase II	
	Concentrate mixture	Concentrate mixture	Concentrate mixture	Concentrate mixture
PART:	1	2	3	4
Groundnut cake	85	25	45	15
Maize	5	60	40	72
Wheat bran	7	12	12	10
Mineral mixture	2	2	2	2
Salt	1	1	1	1

Table 31. Chemical composition of feed ingredients (percent dry matter basis) Phase I

Parameter	Concentrate 1	Concentrate 2	Wheat straw	Green maize
Crude protein	37.07	18.07	4.63	11.86
Ether extract	6.63	3.19	0.65	1.47
Crude fibre	6.54	5.07	38.40	32.47
Total ash	7.68	5.91	11.66	11.28
Nitrogen free extract	42.08	67.76	44.66	42.92
Organic matter	92.32	94.09	88.34	88.72

Table 32. Chemical composition of feed ingredients (percent dry matter basis) Phase II

Parameter	Concentrate 3	Concentrate 4	Wheat straw	Green jowar
Crude protein	24.05	15.53	3.58	6.23
Ether extract	4.98	4.19	0.81	1.93
Crude fibre	5.00	4.84	40.03	32.47
Total ash	9.44	7.38	11.50	9.23
Nitrogen free extract	56.53	68.06	44.08	50.14
Organic matter	90.56	92.62	88.50	90.77

Table 33. Feeding schedule of calves and buffalo calves

Dry matter intake (kg)	Phase I		Phase II	
	HP (Groups 1-4)	OP (Groups 5-8)	OP (Groups 1-4)	LP (Groups 5-8)
Concentrate 1	1.8	-	-	-
Concentrate 2	-	1.8	-	-
Concentrate 3	-	-	2.07	-
Concentrate 4	-	-	-	0.46
Green maize	3.1	3.1	-	-
Green jowar	-	-	5.00	4.20
Wheat straw	2.0	2.0	-	2.25
Total	6.9	6.9	7.07	6.91

respective dietary treatments for a preliminary period of 40 days. Weighed quantity of feed required in a day was offered at 10.00 A.M. Clean water was provided ad lib twice daily to all the animals, i.e., at around 9.30 A.M. and 3.30 P.M., throughout the experiment. After adaptation of animals, a metabolism trial was conducted for 7 days, discarding the collection of the first day. The samples of feed, residue, faeces and urine were collected and analysed for proximate principles as per AOAC (1975).

6.1.5 Infusion studies with ^{15}N-ammonium sulphate and ^{51}Cr-EDTA:

Immediately after the metabolic trial, the animals were shifted to slotted iron cots and an adaptation period of 3 weeks was given. The animals were given the same diet as during the metabolism trial but divided into six equal parts at 2 hourly intervals. After adapting the animals on 2-hourly feeding for 10 days, 6 g $(NH_4)_2SO_4$ of 20 per cent ^{15}N atom excess dissolved in 60 ml distilled water was administered into the rumen of each animal, as a single injection with the help of a funnel attached to a stiff plastic pipe. This $^{15}(NH_4)_2SO_4$ was procured from Rashtriya Chemicals and Fertilizers Ltd., Chembur, Bombay (India). Following this, ^{51}Cr-EDTA (60 µCi in 60 ml dist. water) was also given intra-ruminally to each animal. ^{51}Cr-EDTA was obtained from M/s. Amersham International, U.K. Care was taken to direct the infusions at four different sites in ventral sac of the rumen. Subsequently, the pipe was rinsed thoroughly with water and the washings were poured into the rumen itself.

The rumen liquor samples were obtained through suction process with the help of a plastic tube attached to a stainless steel probe, covered with muslin cloth. The samples thus collected were free from large particulate matter and represented the various places in the rumen at 0 (pre-infusion), 1, 2, 3, 5, 7, 9, 12, 18 and 24 h of infusion. One drop of concentrated sulphuric acid was added in the rumen liquor samples at the time of collection to stop the microbial activity. All the samples were stored in a deep freeze till analysed.

Blood samples were collected in the heparinized test tubes with the help of a hypodermic needle by jugular-venupuncture at 0 (pre-infusion), 1, 2, 3, 5, 7, 9, 12, 18, 24, 36 and 48 h of infusion. Samples were centrifuged at 5,000 rpm for 20 min in a refrigerated centrifuge (IEC model) and plasma was separated. The plasma samples were transferred to a plastic vial and preserved under frozen conditions awaiting analysis.

Aliquots of urine samples were collected at 0, 6, 12, 18, 24, 36, 48, 60 and 72 h and preserved in a deep freeze for further analysis.

6.1.6 Analytical techniques:

6.1.6.1 Assay of ^{51}Cr:

^{51}Cr-EDTA was measured with the help of Packard PRIAS Model PGD Automatic Gamma Scintillation Counter, taking 3 ml of the standard or rumen liquor sample into the counting tubes which fitted into the well of a thallium-activated sodium iodide crystal. Each time the standard

of ^{51}Cr-EDTA solution was run alongwith the unknown samples, in duplicate. The readings were also corrected for the background counts.

Rumen fluid volume and rate of flow of ruminal liquid from the rumen were determined using the values of decrease in radioactivity in the rumen fluid as a function of time after injection of ^{51}Cr-EDTA according to the method of Downes and McDonald (1964).

The natural log specific radioactivity of ^{51}Cr-EDTA was plotted on a graph paper. The curves thus obtained were extrapolated back to zero for getting ^{51}Cr concentration at zero time. From this value and the total amount of marker injected, the volume of rumen fluid and rate of flow were determined as explained below:

$$\text{Rumen volume (l)} = \frac{\text{Dose injected(dpm)}}{\text{Sp. radioactivity at 0 time}}$$

Digesta flow rate l/day= Rumen volume x rate constant x 24

Rate constant = $0.693/t_{\frac{1}{2}}$

Where, $t_{\frac{1}{2}}$ is the time, where the activity (dpm) was half of its original activity (dpm).

6.1.6.2 Estimation of ^{15}N in ruminal ammonia:

^{15}N in ruminal ammonia was determined by the method of Nolan and Leng (1972). For this purpose, 2 ml rumen liquor sample was steam distilled in micro-kjeldahl's distillation apparatus over 10 mg magnesium oxide (which previously had been heated for 3 h at 600°C to decompose any carbonate). Ammonia was collected into 2 per cent

boric acid containing mixed indicator (Bremner, 1965). After titration with N/100 HCl, the distillate was acidified further with dilute hydrochloric acid. This acidified distillate having ammonical-N as ammonium chloride was evaporated to dryness at 70°C (Salter et al., 1979). The dried residues were redissolved in glass distilled water to give a concentration of 1 µg N/µl. About 30 to 40 µl solution of these samples was transferred to capillary tubes with the help of a Hamilton micro-syringe, and were dried at 70°C. These capillary tubes were transferred to discharge tubes containing copper oxide wire (1-2 mg) and calcium oxide (3-6 mg). Discharge tubes were sealed in a sample preparation unit (Plate 1) (Jasco Model DPS-10M for NIA-I) under vacuum, 10^{-3} Torr (Pirani Gauge Model PG-3A, Wakaida Sci. Instruments, Japan). Sealed discharge tubes were heated at 550°C for 3 h in a furnace for the conversion of ammonium chloride to pure N_2 gas as per the following reaction:

$$2NH_4Cl + 4\ CuO \xrightarrow[3\ h]{550\,^{\circ}C} N_2 + 2\ Cu + 2\ CuCl + 4H_2O$$

This whole process was based on Dumas dry combustion method as described by Bremner (1965). The copper oxide (CuO) had been previously heated at 550°C for 3 to 4 h and the calcium oxide (CaO) at 1,000°C for a similar period.

^{15}N-isotopic abundance was determined by scanning the sealed samples of pure N_2 gas in optical emission spectrometer ^{15}N-analyser, JASCO Model NIA-I N-15 Analyser, Japan Spectroscopic Co.Ltd., Tokyo, Japan (Plate 2).

To prevent cross-contamination, 30 ml absolute alcohol was distilled between samples to remove traces of ammnia which were adsorbed on to the glassware of the distillation apparatus (Bremner, 1965).

6.1.6.3 ^{15}N in rumen liquor suspended bacteria:

Bacteria from the rumen liquor samples were separated by the method of Salter et al. (1979). The samples of rumen fluid were first strained through 4 layers of muslin cloth to remove coarse particulate material. The strained rumen fluid was diluted with saline (2:1) and centrifuged at low speed (2,000 rpm) for 10 min in a refrigerated centrifuge (IEC Model) to remove feed particles. The supernatant fluid was then centrifuged at 14,000 rpm for 10 min to produce a sediment of crude bacteria. This sediment was washed twice by resuspension in saline and repeating the centrifugation, then finally washed by resuspension in distilled water followed by centrifugation. The pure bacterial pellets were subjected to kjeldahl digestion by adding 3 ml of concentrated H_2SO_4 and catalyst (1.20 g potassium sulphate, 0.05 g mercuric oxide). After completion of digestion and cooling, the digested samples were diluted to 10 ml with water. Portion of the diluted samples were steam distilled using NaOH as the alkaline reagent. Ammonia-N was collected in a boric acid solution containing mixed indicator and then titrated and acidified for the measurement of ^{15}N-abundance as described earlier.

6.1.6.4 ^{15}N in plasma urea:

^{15}N-abundance in plasma urea-N was determined by

the method of Nolan and Leng (1972). The plasma samples (5 ml) were deproteinized with successive additions of 10 ml water, 5 ml 10 per cent (w/v) sodium tungstate and 5 ml 0.33 M sulphuric acid. After centrifugation at 5,000 rpm for 10 min, the clear supernatant liquid was transferred to a culture tube and buffered to pH 6.5. This was incubated with urease enzyme (E.C. 3.5.1.5, 1 unit liberated 1.0 mg of ammonia-N from urea, procured from Sigma Chemicals, U.S.A.) at 37°C overnight. Urease-enzyme used in this study had no detectable ammonia. The buffered filterate containing urease was subjected to distillation for ammonia as described previously.

6.1.6.5 ^{15}N in urine samples:

^{15}N in total urinary-N was determined as per the normal kjeldahl procedure using NaOH as the alkaline reagent during steam distillation. Samples were processed and ^{15}N-abundance was determined according to the method described earlier.

6.1.7 $^{14}N/^{15}N$ ratio determination by Emission Spectrometry:

6.1.7.1 Principle of the detection method:

The analysis of ^{15}N by the emission spectrometric technique depends upon the property of nitrogen gas at relatively low pressure to emit light of characteristic wave lengths when excited by radio or micro waves. Gaseous molecules are excited to higher energy levels and on returning to the unexcited state emit energy over a wide range of frequencies. Emission spectrum of nitrogen which is

composed of a succession of bands as shown in Fig. 12. The presence of an extra neutron in the ^{15}N nucleus causes shifts in the energy transitions of ^{15}N containing molecule which results in change in the wave lengths of each of the band heads.

6.1.7.2 Choice of the bands for the analysis:

The 2-0 transition shows a proncunced shift resulting in readily measurable displacement of the band heads which normally appear in the ultra-violet region of 297.7 nm for $^{14}N\ ^{14}N$ molecule, 298.3 nm for $^{14}N\ ^{15}N$ and 298.8 nm for $^{15}N\ ^{15}N$. The reasons for this choice were that for this transition, the shifting of the isotopic molecule bands and their intensity have an optimum effect, and the disturbing bands from possible impurities have a minimum effect (Leicknam et al., 1968). The intensities of the bands are proportional to their nitrogen content (Fig. 13) so that in an equilibrium mixture of the three molecular forms of nitrogen, the proportion of ^{15}N can be calculated from the ratio of the intensities of the bands of mass ^{28}N and ^{29}N.

6.1.7.3 Conversion of nitrogen into nitrogen gas:

Sample nitrogen can be converted to gaseous nitrogen by various methods (Fig. 14). Generally, biological samples are converted to ammonium sulphate or ammonium chloride by kjeldahl digestion, followed by steam distillation of ammonia from the digested sample under alkaline conditions. Ammonium sulphate or ammonium chloride is converted

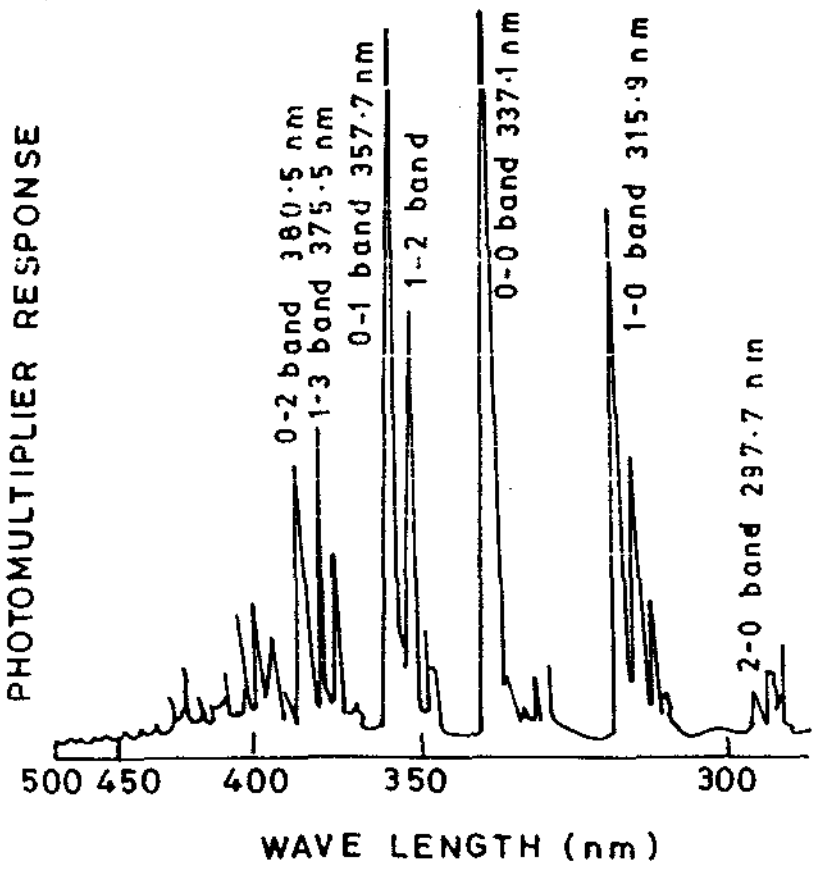


FIG.12. EMISSION SPECTRUM OF NATURAL NITROGEN (0·37 atoms % ^{15}N)

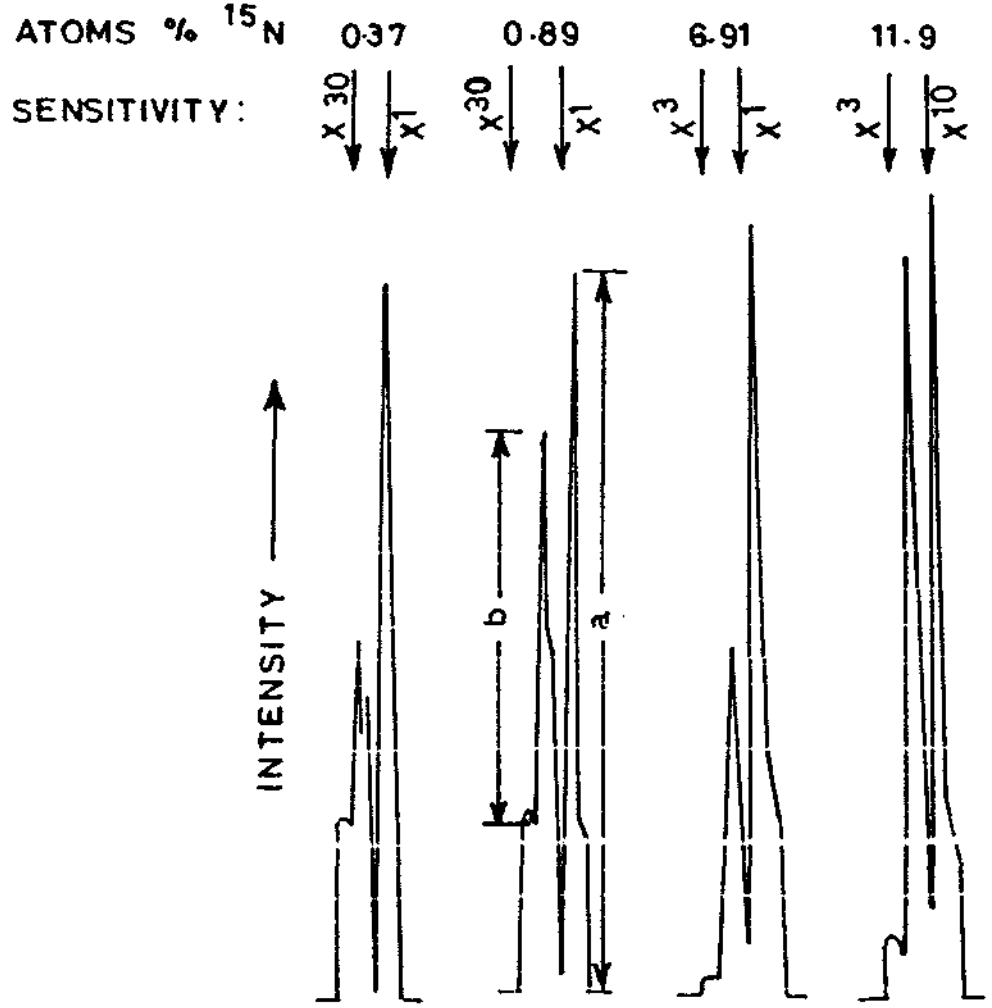


FIG.13. EMISSION SPECTRUM OF NATURAL AND ^{15}N ENRICHED NITROGEN (2-0 TRANSITION)

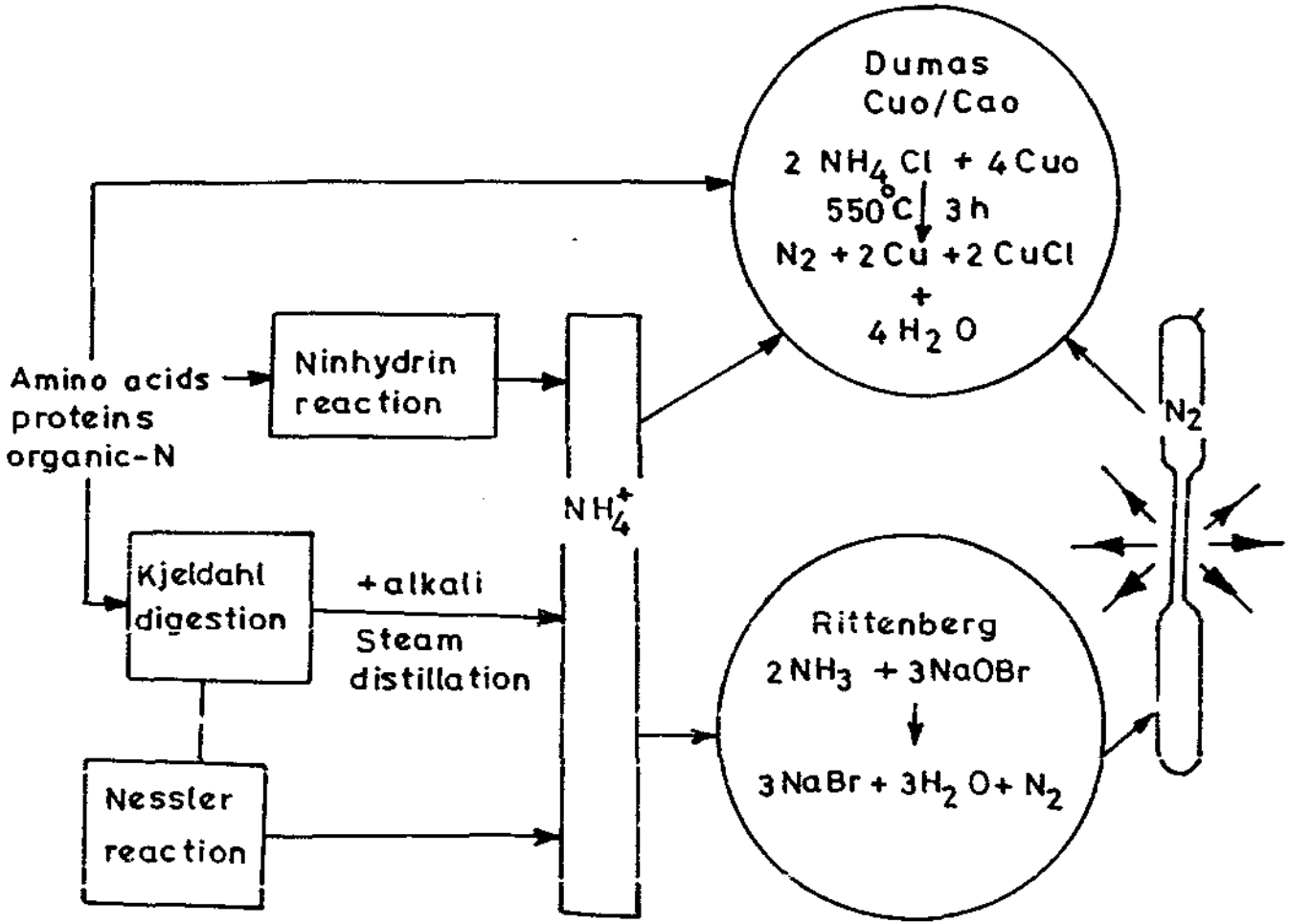


FIG. 14. PREPARATION OF N_2 EMISSION TUBE FOR ISOTOPE ANALYSIS

under vacuum to nitrogen gas by (i) oxidation of ammonia with an alkaline potassium hypobromide solution, known as the Rittenberg method or (ii) by dry combustion of the sample in the discharge tube with copper oxide and calcium oxide (Dumas method). In the present study, Dumas method was used for converting nitrogen to its gaseous form.

6.1.8 Analysis of recorded ^{15}N spectrum:

The emission band spectra separated by the spectrophotometer are automatically recorded on the graph. The spectra are recorded with constant gain for $^{14}N_2$ and varied gains for $^{14}N\,^{15}N$. For calculation of ^{15}N ratio, when it is 70 per cent or less, it is derived from the measured value of $^{14}N_2$ and $^{14}N^{15}N$ using the formula-I, and when the ratio is higher than 70 per cent formula-II is used with the data of $^{14}N^{15}N$ and $^{15}N_2$.

$$^{15}N \text{ (atom \%)} = \frac{100}{2R + 1} \qquad \text{.....I}$$

where,

$$R = M \frac{I_{28}}{I_{29}}$$

$$M = x_1, x_2, x_4, x_8, x_{16}, x_{32} \text{ and } x_{64}$$

$$^{15}N \text{ (atom \%)} = \frac{100}{R'/2 + 1} \qquad \text{.....II}$$

where,

$$R' = \frac{I_{29}}{I_{30}}$$

I_{28}= intensity of $^{14}N_2$ spectrum

I_{29} = intensity of $^{14}N^{15}N$ spectrum

I_{30} = intensity of $^{15}N_2$ spectrum

6.1.9 Preparation of standard curve:

Standard curve was prepared by using "Heavy nitrogen gas standards No. 0111" supplied by Hikari Kogyo Co. Ltd., Tokyo, Japan. Samples of ^{15}N enrichment were corrected with the standard curve to give net ^{15}N atom per cent excess.

6.1.10 Mathematical procedure:

6.1.10.1 Analysis of isotope ratio with time curve in primary compartment:

A curve was plotted for ^{15}N-abundance or radio specific activity versus time. In single injection technique used in this study, the equation for the lines which were fitted to the enrichment/radioactivity values obtained at any time for sample taken from a samples pool were of the general form as indicated below:

$$Et \text{ or } SR_t = \sum_{i=1}^{n} Aie^{-mit}$$

where,

t = time in hour

Et = enrichment of N (atom % excess)

SR_t = specific radioactivity at time t

A = zero time intercept of each exponential component

m = rate constant of each component

n = number of exponential component

(i) = exponential component identification

Multi-exponential curves were fitted by the usual "peeling process" as described by White et al. (1969). The terminal exponential to the curve against time was fitted by eye or least square method,and then by subtraction of this line from the earlier part of the curve, a second curve was obtained, a second exponential was drawn and the procedure repeated. In general, two or three exponential components were obtained.

From the above equation, the following calculations were done as described by Nolan and Leng (1974):

6.1.10.2 Size (Q) of the primary compartment:

$$Q = \frac{P}{\sum_{i=1} A_i}$$

where,

P = number of ^{15}N atoms injected

6.1.10.3 Total entry rate (E):

It is the rate (mass ÷ unit time) at which all traces enter and leave the primary compartment which is in steady state. Hence, in the present study, total entryy rate (E) was calculated as per the follcwing equation:

$$E = Q\sum_{i=1}^{n} A'_i m_i$$

where,

A'_i are fractional zero time intercepts, e.g.

$$A'_2 = \frac{A_2}{\sum_{i=1}^{n} A_i}$$

and, therefore,

$$\sum_{i=1}^{n} A'_i = 1$$

6.1.10.4 Irreversible loss rate (ILR):

It is the fraction of the total entry rate (mass $\frac{}{}$ unit time) through the primary compartment that leaves but do not return during the experimental period.

$$ILR = \frac{P}{\sum_{i=1}^{n} \frac{A_i}{m_i}}$$

6.1.10.5 Recycling rate:

Recycling rate is defined as that part of the total entry which leaves the compartment and returns to it during the experimental period and is calculated as:

Recycling rate = total entry rate - irreversible loss rate

6.1.10.6 The area (X) under fitted multi-exponential curves was determined by the method of Nolan et al. (1976), i.e.

$$X = \sum_{i=1}^{n} \frac{Ai}{mi}$$

6.1.10.7 The proportion(Q) of the nitrogen in any secondary pool (S) derived from primary pool (P) was given by:

$$Q = \frac{\text{Area under the enrichment curve for pool S}}{\text{Area under the enrichment curve for pool P}}$$

6.2 RESULTS AND DISCUSSION

PHASE I

6.2.1 Effect of aflatoxin on digestibility of nutrients:

Effect of aflatoxin on digestibility of nutrients fed HP and OP diets is given in Table 34. The digestibility coefficients of DM, OM and crude protein were in Group 1: 69.08, 72.62, 71.37; Group 2: 64.45, 68.03, 73.85; Group 3: 69.70, 72.46, 73.86; Group 4: 67.60, 71.21, 73.48; Group 5: 63.27, 66.03, 64.43; Group 6: 66.29, 69.44, 64.93; Group 7: 69.22, 72.75, 69.06 and Group 8: 68.18, 72.16, 68.13. The results indicated that aflatoxin had no effect on the digestibility of these nutrients. In pigs, however, Hale and Wilson (1979) reported low digestibility coefficients of DM and CP fed aflatoxin contaminated corn. The digestibility of crude protein was more in both the species fed higher level of protein. Results also indicated that there was no species difference in the digestibility of crude proteins, as also observed in the earlier experiments (Dhiman and Arora, 1985). The digestibility of crude fibre, ether extract and NFE was alike in all the groups.

6.2.2 Effect of aflatoxin on nitrogen balance:

The data on nitrogen balance given HP (Groups 1-4) and OP (Groups 5-8) levels are presented in Table 35. The total nitrogen intake in 8 groups was 179.8, 179.8, 179.8, 178.0, 126.5, 125.46, 126.0, 125.5 g/day respectively.

Table 34. Effect of aflatoxin on digestibility coefficient of nutrients fed HP and OP diets

Group	Attribute					
	DM	OM	CP	CF	EE	NFE
1	69.08	72.62	71.37	60.40	75.20	68.95
2	64.45	68.03	73.85	59.80	74.29	69.13
3	69.70	72.46	73.86	62.15	75.05	72.36
4	67.60	71.21	73.48	62.00	73.25	68.71
5	63.27	66.03	64.43	59.50	70.55	66.51
6	66.29	69.44	64.93	60.20	68.35	68.85
7	69.22	72.75	69.06	63.50	66.98	71.09
8	68.18	72.16	68.13	64.40	69.14	68.77

Table 35. Effect of feeding aflatoxin on nitrogen balance fed HP and OP diets

Attribute	GROUP							
	1	2	3	4	5	6	7	8
Live weight (kg)	325.00	387.50	369.00	337.00	335.00	360.00	372.00	370.00
N intake (g/d)	179.80	179.80	179.80	178.00	126.50	125.46	126.00	125.50
N excreted in faeces (g/d)	51.47	47.00	47.00	47.20	45.00	44.00	39.00	40.00
N absorbed (g/d)	128.39	132.80	132.80	130.80	81.50	81.46	87.00	85.50
N excreted in urine (g/d)	99.26	106.81	93.80	105.40	43.40	48.50	29.10	41.20
Total N excreted (g/d)	150.73	153.81	140.80	152.60	88.40	92.50	68.10	81.20
N retained (g/d)	29.07	25.99	39.00	25.40	38.10	32.96	57.90	44.30
N retained as percent of absorbed	22.64	19.57	29.37	19.42	46.75	40.46	66.55	51.81
N retained as percent of absorbed per kg live weight	0.070	0.051	0.079	0.058	0.139	0.112	0.179	0.140
N digestibility (%)	71.37	73.85	73.86	73.48	64.43	64.93	69.06	68.13
N retained as percent of intake	16.17	14.45	21.69	14.27	30.12	26.27	45.95	35.30
N excreted in faeces as percent of intake	28.63	26.14	26.14	26.52	35.58	35.07	30.95	31.87
N excreted in urine as percent of intake	55.20	59.40	52.17	59.24	34.30	38.66	23.09	32.83

The average N intake was 179.35 and 125.86 g/day respectively in HP and Op fed groups, which indicated an average of 42.5 per cent more N intake l in groups 1 to 4. The nitrogen (g/day) excreted in faeces was: Group 1: 51.47, Group 2: 47.0, Group 3: 47.0, Group 4: 47.2, Group 5: 45.0, Group 6: 44.0, Group 7: 39.0 and Group 8: 40.0. The percent nitrogen excretion through faeces of the intake was: Group 1: 28.63, Group 2: 26.14, Group 3: 26.14, Group 4: 26.52, Group 5: 35.58, Group 6: 35.07, Group 7: 30.95, and Group 8: 31.87. These results indicated that there was less nitrogen excretion through faeces at higher levels of nitrogen intake in both the species as even observed earlier by Dhiman and Arora (1985), but not affected by 1 ppm aflatoxin level. This level of aflatoxin was not also effective in kids as observed earlier in this laboratory (Sinha, 1983). But Lynch et al. (1973) observed less N excretion through faeces in young calves fed graded levels (0.0 - 0.8 mg/kg body weight) of aflatoxin as compared to control.

The quantity of N excreted (g/day) through urine was: Group 1: 99.26, Group 2: 106.81, Group 3: 93.8, Group 4: 105.4, Group 5: 43.4, Group 6: 48.5, Group 7: 29.1 and Group 8: 41.2. These results indicated that excretion of N through urine was more in animals fed higher amount of nitrogen as compared to groups 5-8. Results also indicated that excretion of N through urine was more as a result of aflatoxin feeding in calves of both the species. Lynch et al. (1973) and Hale and Wilson (1979) also noted more nitrogen excretion in calves and pigs respectively

as a result of aflatoxin feeding. The percent N excretion through urine of the intake was: Group 1: 55.2, Group 2: 59.40, Group 3: 52.17, Group 4: 59.24, Group 5: 34.30, Group 6: 38.66, Group 7: 23.09 and Group 8: 32.83. Nitrogen retention (g/d) was: Group 1: 29.07, Group 2: 25.99, Group 3: 39.00, Group 4: 25.40, Group 5: 38.10, Group 6: 32.96, Group 7: 57.90 and Group 8: 44.30. Nitrogen retention data indicated that all the animals were in positive nitrogen balance, although the quantity of N retained was less in aflatoxin fed animals. This trend was observed in calves of both the species fed HP and OP diets. Results also indicated that N balances (g/d) were more in buffalo calves as compared to crossbred calves. Further, N balances were more with OP level as compared to animals fed HP level. The amount of N retained as percent of absorbed N as such and when calculated on per kg live weight basis were: Group 1: 22.64, 0.070; Group 2: 19.57, 0.051; Group 3: 29.37, 0.079; Group 4: 19.42, 0.058; Group 5: 46.75, 0.139; Group 6: 40.46, 0.112; Group 7: 66.35, 0.179 and Group 8: 51.81, 0.140 g/day respectively. The data indicated that the values of these parameters were more in animals fed OP than HP groups and buffalo calves seemed to be superior to crossbred calves, when compared on the basis of these parameters. In aflatoxin fed animals, N retention as percent of absorbed was uniformly less in all the groups per unit live weight. Lynch et al. (1973) also observed a decrease in N retention as percent of absorbed in calves given graded levels (0 - 0.8 mg/kg body weight) of aflatoxin.

6.2.3 Effect of aflatoxin on ruminal NH_3-N concentration, water and ammonia outflow from the rumen fed HP and OP diets

The ruminal ammonia-N concentration at different hours in calves of two species is presented in Table 36. The mean ruminal ammonia-N concentration (mg/100 ml SRL) was higher in HP fed animals irrespective of species and aflatoxin intake. It appeared as if 1.0 ppm aflatoxin in the diet of these animals did not decrease the rate of fermentation in the rumen which might be due to microbial detoxification (Mathur et al., 1976) or high level of protein giving a protective effect against aflatoxin toxicity (Balaraman and Arora, 1984).

Estimates of rumen fluid volume and water flow rates to the lower digestive tract determined with the help of ^{51}Cr-EDTA, using isotopic dilution techniques are given in Table 37. The flow of ammonia-N (g/day) to the lower digestive tract in different treatments was calculated by multiplying the mean ruminal ammonia-N (mg/l) concentration with fluid flow rates (l/day). The rumen volumes (l) and their values as percent of the body weight in different groups were: Group 1: 37.70, 11.60; Group 2: 56.00, 14.45; Group 3: 45.38, 12.29; Group 4: 55.49, 16.47; Group 5: 45.71, 13.64; Group 6: 54.90, 15.25; Group 7: 50.46, 13.56 and Group 8: 43.77, 11.82. Results indicated that rumen volumes were not influenced either due to aflatoxin intake, or species difference or level of protein. Similar results were reported by Arora (1987), in cattle

Table 36. Effect of feeding aflatoxin on ruminal NH_3-N (mg/100 ml SRL) concentration fed HP and OP diets

Group	Hours after isotope injection										Average ± S.E.
	0	1	2	3	5	7	9	12	18	24	
1	20.0	21.0	23.0	28.0	32.0	26.0	22.0	11.0	21.0	18.0	22.2 ± 1.81
2	17.0	27.0	25.0	33.0	23.0	29.0	27.0	26.0	17.0	19.0	24.3 ± 1.67
3	19.0	22.0	23.0	27.0	39.0	37.0	28.0	23.0	23.0	22.0	26.3 ± 2.11
4	15.0	23.0	26.0	27.0	33.0	30.0	22.0	22.0	19.0	18.0	23.5 ± 1.76
5	7.0	9.0	10.0	13.0	18.0	12.0	11.0	10.0	9.0	8.5	10.7 ± 0.98
6	10.0	11.0	16.0	17.0	20.0	16.0	17.0	10.0	10.0	10.0	13.7 ± 1.22
7	5.0	9.0	10.0	12.0	15.0	15.0	11.0	10.0	9.0	8.0	10.4 ± 0.97
8	7.0	10.0	13.0	14.0	20.0	12.0	10.0	10.0	9.0	8.0	11.3 ± 1.18

Table 37. Effect of aflatoxin intake on water and ammonia flow from rumen fed HP and OP diets

Group	Body weight (kg)	Ruminal NH_3-N concentration (mg/l)	Rumen volume (l)	Rumen fluid outflow rate (l/d)	Ammonia outflow with rumen fluid (g N/d)*	Rumen volume (l) % body wt.
1	325.0	223.0	37.70	87.34	19.39	11.60
2	387.5	243.0	56.00	90.20	21.87	14.45
3	369.0	263.0	45.38	105.90	27.85	12.29
4	337.0	235.0	55.49	123.70	29.07	16.47
5	335.0	107.5	45.71	93.95	10.09	13.64
6	360.0	137.0	54.90	88.14	12.08	15.25
7	372.0	104.0	50.46	89.88	9.35	13.56
8	370.0	113.7	43.77	107.18	12.18	11.82

* Calculated as the product of outflow (l/d) and measured mean ammonia-N concentration (g/l) in rumen fluid

and buffalo calves kept under dietary conditions similar to this experiment.

The ammonia-N outflow from rumen fluid in different groups was: Group 1: 19.39, Group 2: 21.87, Group 3: 27.85, Group 4: 29.07, Group 5: 10.09, Group 6: 12.08, Group 7: 9.35 and Group 8: 12.18, g/day. The outflow of ammonia-N was more in HP fed groups 1-4, which was related to higher nitrogen intake and as a result of greater ruminal NH_3 concentration. It is no different to reports in sheep (Nolan et al., 1976; Nolan and Stachiw, 1979). The NH_3 outflow rate from rumen was 2.85 g N/day when daily nitrogen intake was 16.3 g, but it was reduced to 0.41 g N/day when the nitrogen intake was only 6.2 g/day.

6.2.4 Effect of aflatoxin on the kinetics of ammonia in rumen and blood fed HP and OP diets:

The change at different time intervals in ^{15}N-enrichment of NH_3-N in the rumen fluid and enrichment of rumen bacterial-N, plasma urea-N and urinary-N after single intra-ruminal injection of ^{15}N-ammonium sulphate fed HP and OP diets with or without aflatoxin are depicted in Figs. 15 to 22.

The enrichment of rumen NH_3-N as a function of time after the intra-ruminal injection of ^{15}N-ammonium sulphate was best described by a curve with two exponential components. One component described the rapid decline in enrichment from hours 1 to 5 after injection. The other component was described by slower decline in enrichment from 5 to 24.

FIG. 15. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

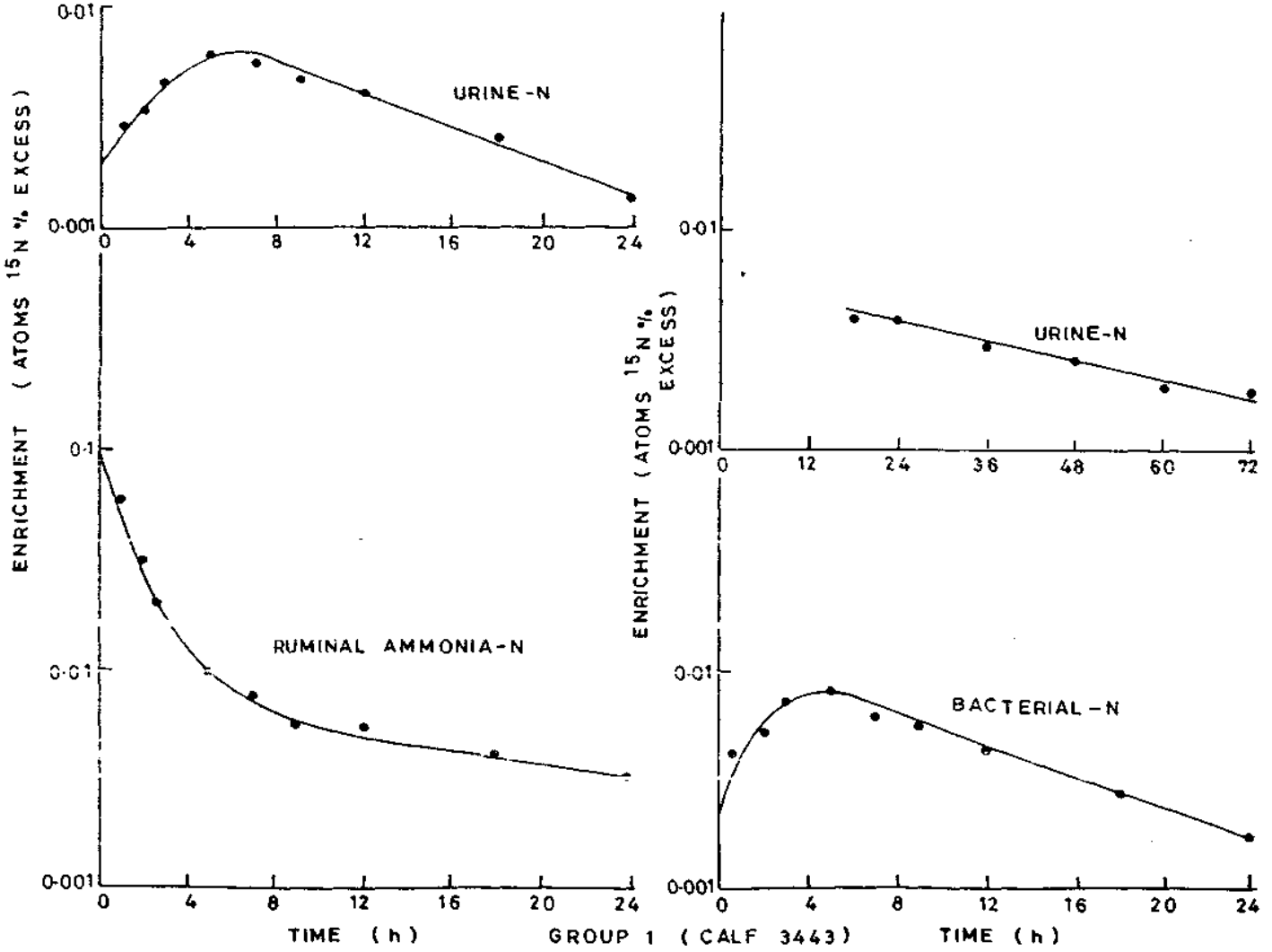


FIG. 16. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

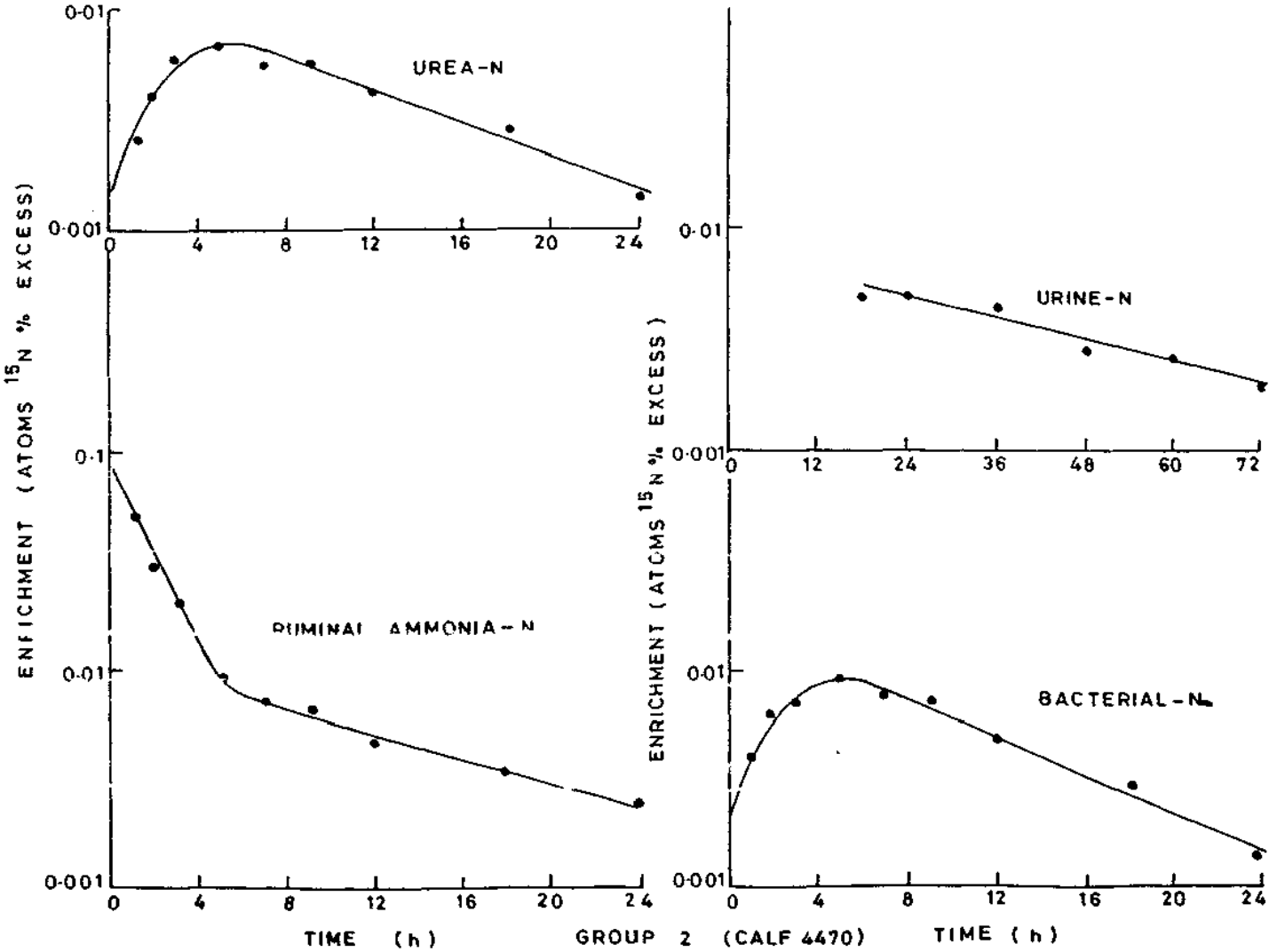


FIG.17. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

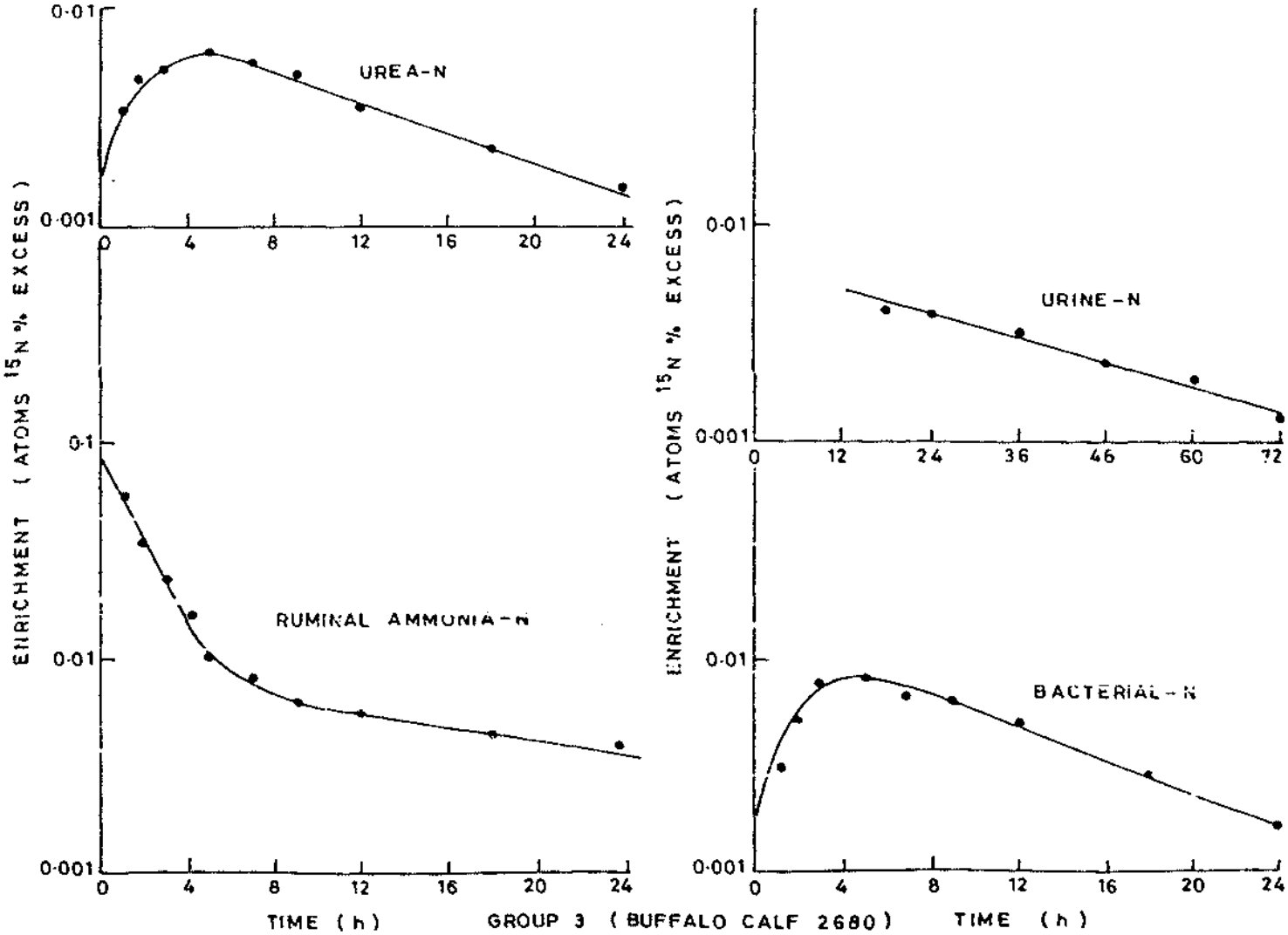


FIG .18. ENRICHMENT OF RUMINAL AMMONIA-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

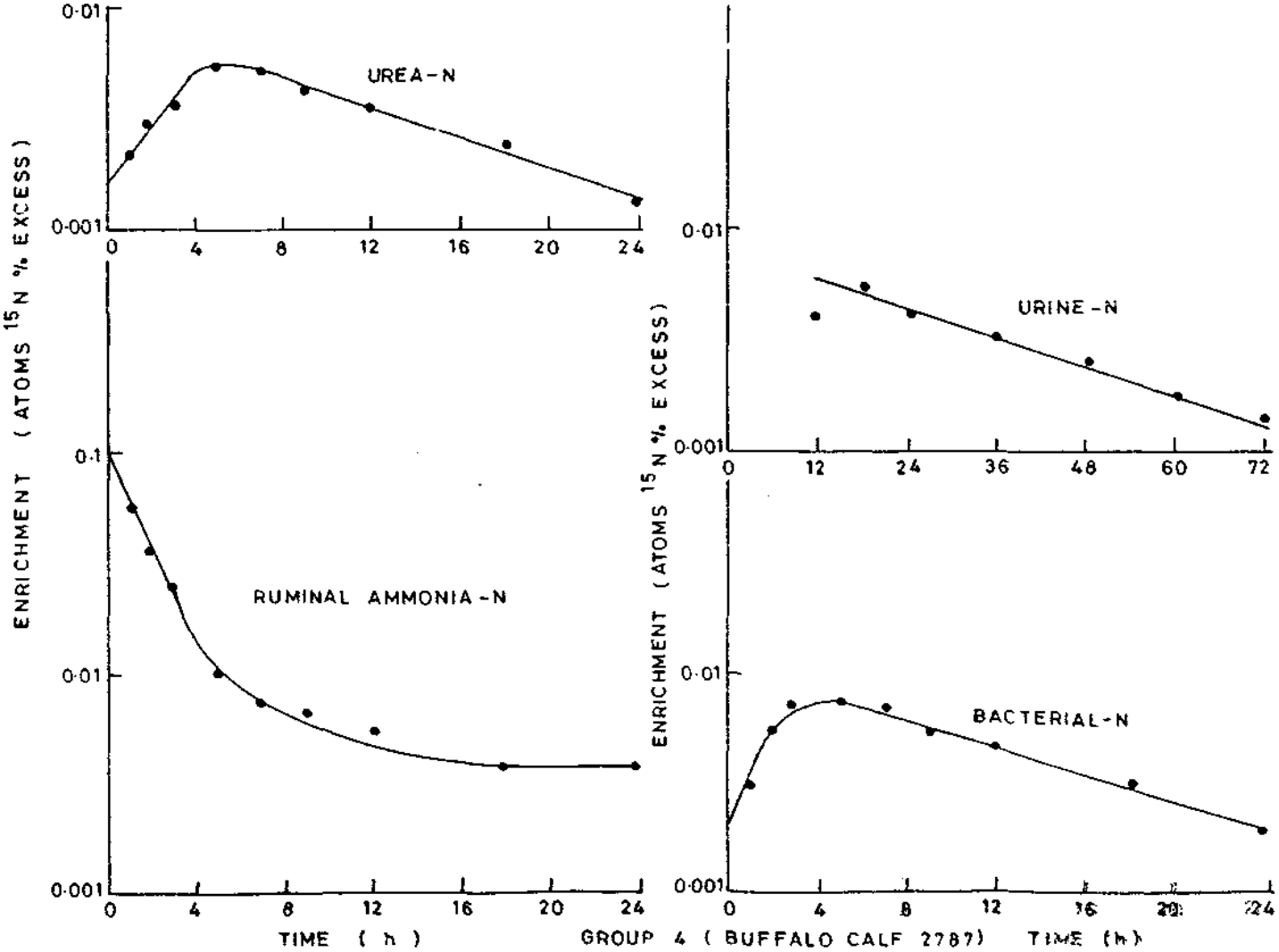


FIG.20. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

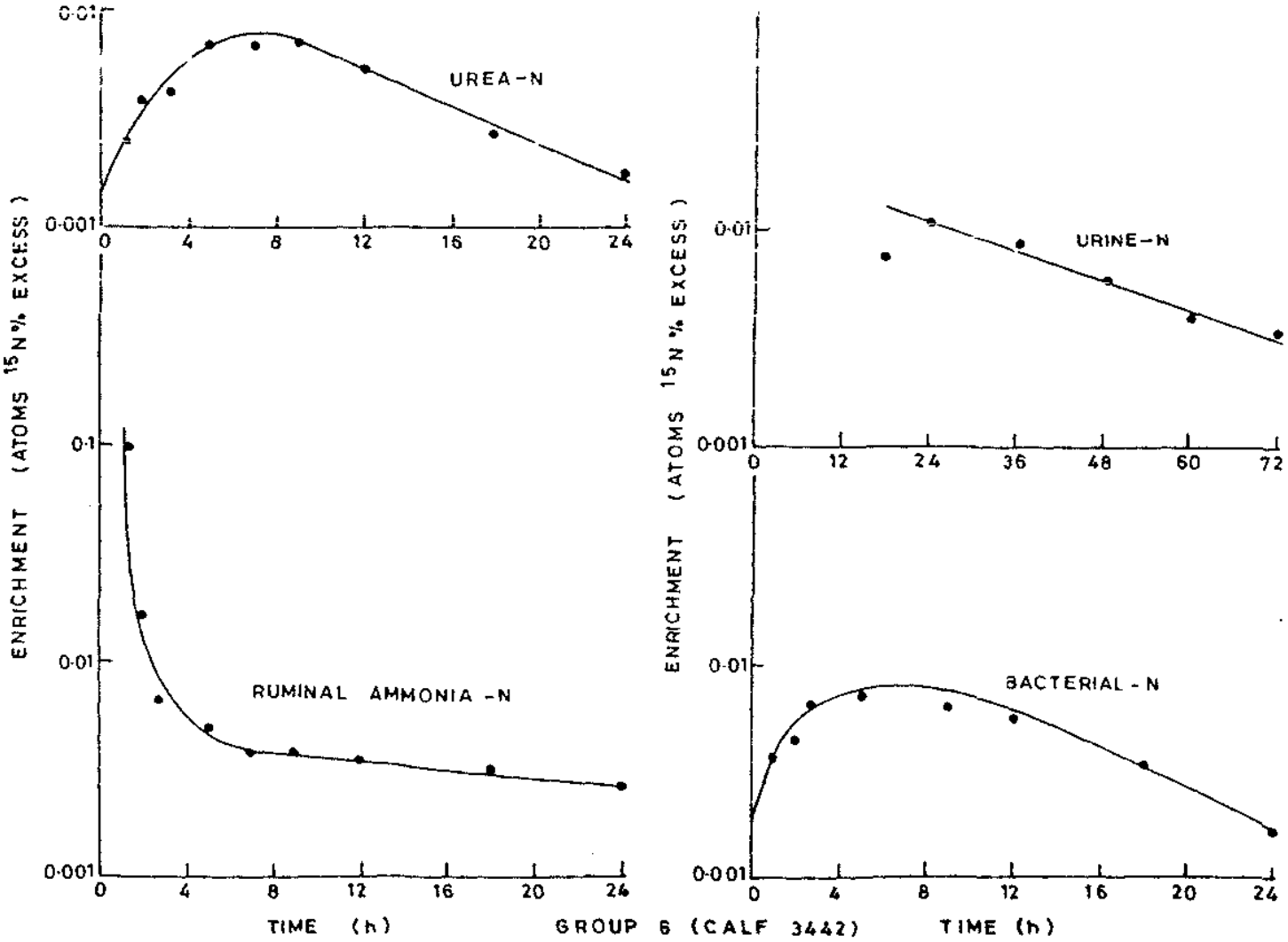


FIG.21. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

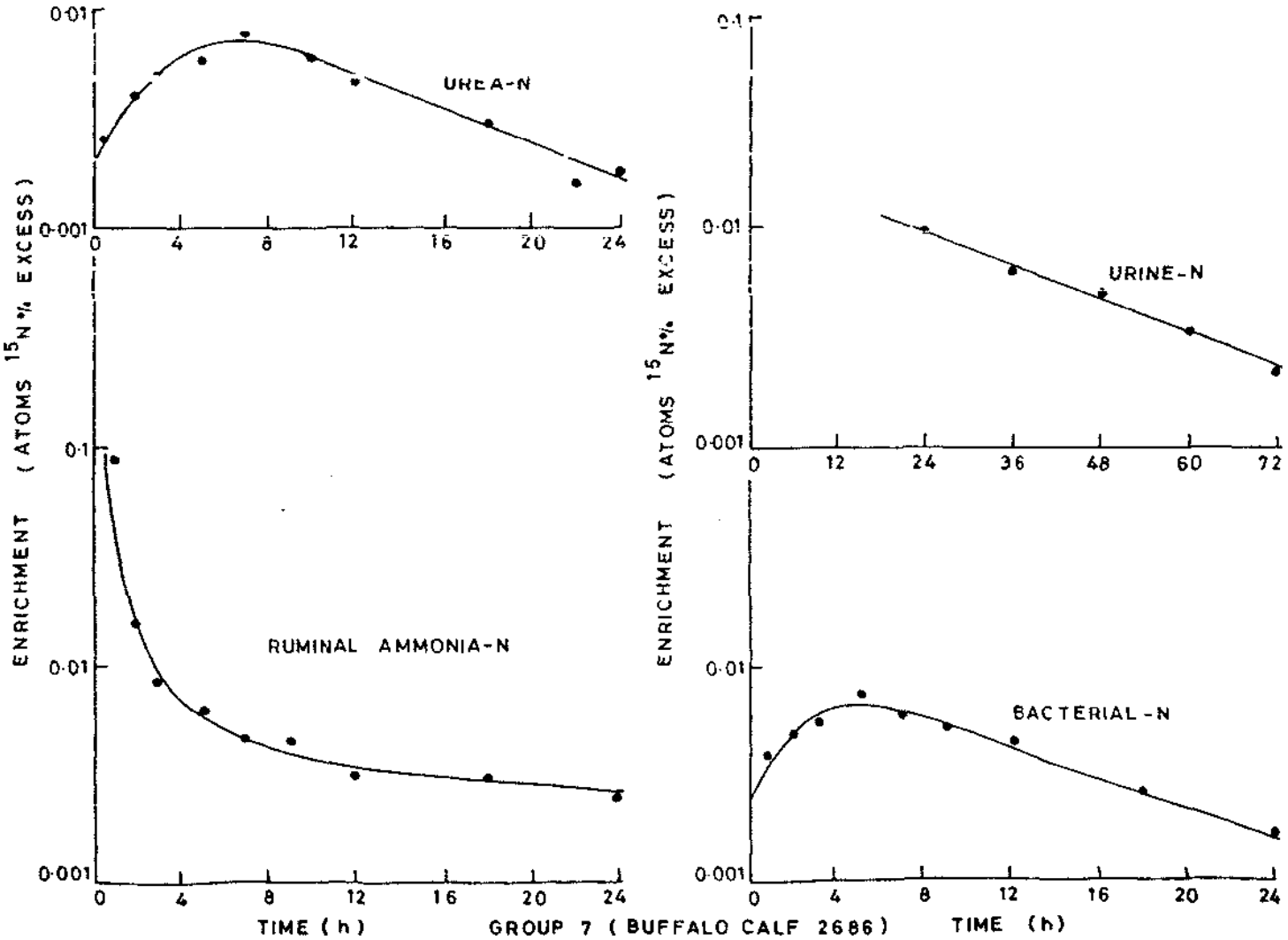
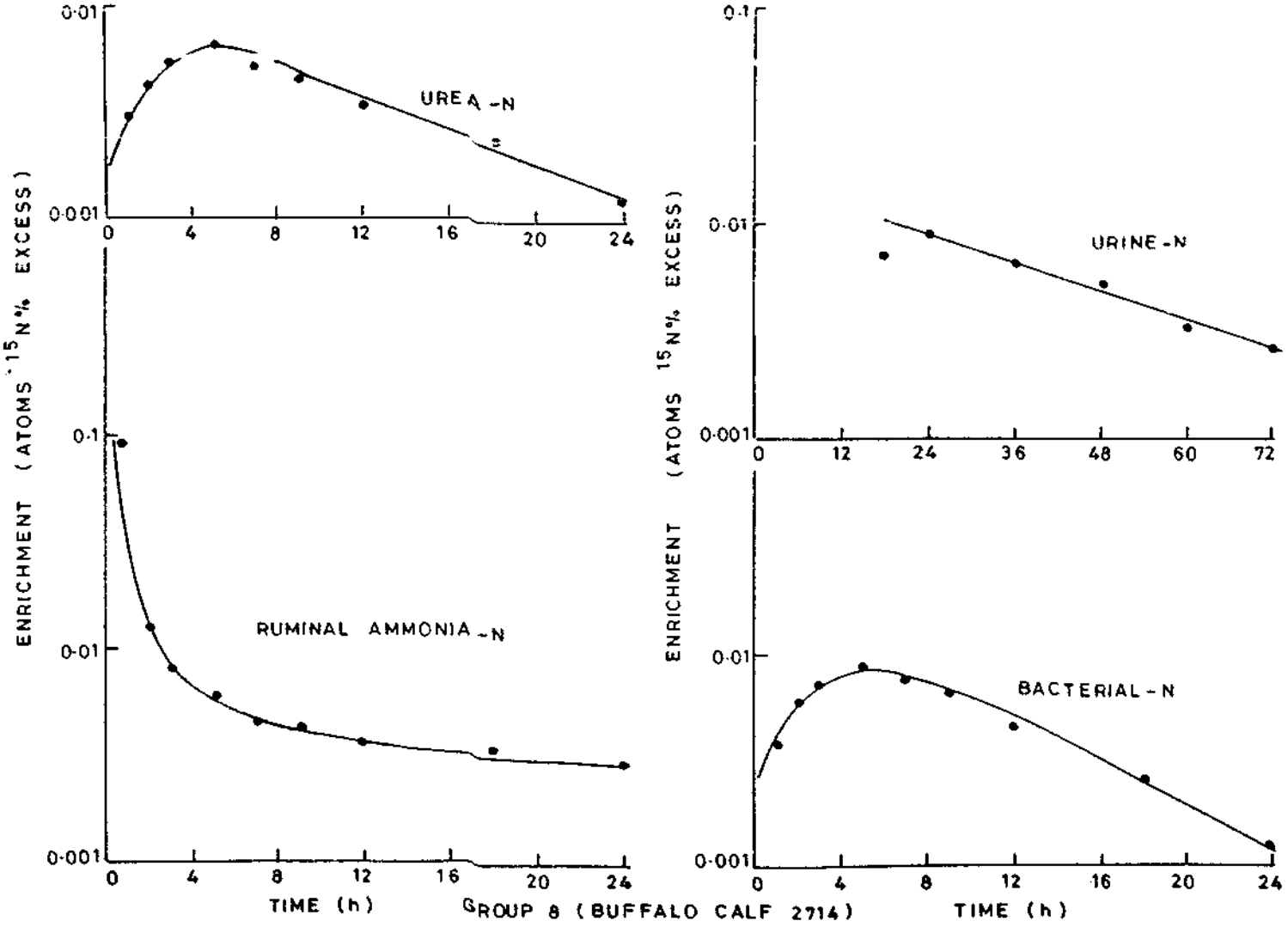


FIG 22. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE



GROUP 8 (BUFFALO CALF 2714)

Similarly, ^{15}N-enrichment in bacterial and plasma urea-N was also reflected by the curves with two exponential components. In case of plasma urea-N, the first component showed a rapid increase from 0 to between 3 to 5 hours after isotope injection. The second component indicated a gradual decline subsequently upto 24 hour. However, enrichment of ^{15}N in urinary-N was described by single exponent.

The above mentioned exponents were used to calculate the size of the ammonia pool sampled from the rumen and its total entry rate, irreversible loss rate and recycling rate of ammonia which have been presented alongwith data on bacteria-N, plasma urea-N and urinary-N derived from ruminal ammonia in Table 38.

The values of ruminal-NH_3 pool size in different groups were: Group 1: 4.63, Group 2: 3.96, Group 3: 4.87, Group 4: 4.32, Group 5: 2.90, Group 6: 2.80, Group 7: 2.83 and Group 8: 3.06 g nitrogen. The size of ammonia pool was more in HP fed groups 1-4 as expected, irrespective of species. Since the values in control and aflatoxin fed animals did not differ, it appeared as if the present level of aflatoxin had no effect on rumen microbes under such dietary feeding conditions.

Ruminal-NH_3 entry rates were 27.35, 30.36, 37.12, 36.47, 18.26, 21.23, 20.21, 19.76 g N/day in 8 groups respectively. The higher rates of ammonia production in first 4 groups were due to higher N intake as compared to other 4 groups (5-8). Species difference and aflatoxin

Table 38. Kinetics of ammonia in rumen and blood given aflatoxin with two levels of protein, using sinlge injection of ^{15}N-ammonium sulphate (Phase I)

Group	Rumen NH_3 pool size (g N)	Ruminal NH_3 entry rate (g N/d)	Ruminal NH_3 irreversible loss rate (g N/d)	NH_3 irreversible loss rate as (%) of entry rate	Recycling rate (g N/d)	Suspended bacterial-N derived from ruminal ammonia (%)	Plasma urea-N derived from ruminal NH_3 (%)	Urinary-N derived from ruminal ammonia (%)
1	4.63	27.35	19.01	69.50	8.34	42.85	36.38	34.15
2	3.96	30.36	21.35	70.32	9.01	38.41	39.17	38.20
3	4.87	37.12	25.77	69.40	11.35	38.62	39.45	20.42
4	4.32	36.47	26.91	73.79	9.56	33.75	38.40	25.44
5	2.90	18.26	12.59	68.95	5.67	49.81	32.20	20.57
6	2.80	21.23	14.09	66.37	7.14	50.62	30.25	23.85
7	2.83	20.21	12.74	63.04	7.47	47.24	29.40	16.41
8	3.06	19.76	13.25	68.37	6.51	49.42	35.85	19.80

feeding had no effect on rumen ammonia entry rate.

The irreversible loss rates of ammonia-N in different groups were: Group 1: 19.01, Group 2: 21.35, Group 3: 25.77, Group 4: 26.91, Group 5: 12.59, Group 6: 14.09, Group 7: 12.74 and Group 8: 13.25 g N/day. These results again showed higher irreversible loss rates in groups 1-4 than the animals of groups 5-8. When the irreversible loss rates of ammonia-N were calculated as percent of the ruminal-NH_3 entry rates, the values were 69.50, 70.32, 69.40, 73.79, 68.95, 66.37, 63.04 and 68.37 in groups 1 to 8 respectively, showing uniform positive correlation (Nolan and Leng, 1972; Nolen et al., 1976; Kennedy and Milligan, 1978a). The latter workers fed lucerne hay or brome grass pellets to sheep giving irreversible loss rate of ruminal NH_3 to vary from 61.00 to 69.70 percent of the total entry rate. Dhiman and Arora (1985) reported irreversible loss rates of ruminal-NH_3 ranging from 57.52 to 73.53 per cent in cattle and buffalo calves fed optimum and sub-optimum protein diets.

Recycling of ammonia to the rumen-NH_3 pool is a continuous process. The recycling of ammonia might take place within the rumen itself (Nolan and Leng, 1972) or by way of plasma urea transfer to the rumen (Kennedy and Milligan, 1978b). The rates of recycling of ammonia to the rumen-NH_3 pool were: 8.34, 9.01, 11.35, 9.56, 5.67, 7.14, 7.47 and 6.51 g N/day in groups 1 to 8 respectively. Earlier, higher recycling rates of ammonia-N to the rumen ammonia pool given optimum protein in both the species

were observed as compared to sub-optimum protein diets (Dhiman and Arora, 1985).

Ammonia is produced in the rumen as a result of deamination of various amino acids, formed during the process of proteolysis of dietary proteins by rumen microbes. A significant amount of ammonia produced during this process is utilized by the rumen microbes for their cellular protein synthesis. Unutilized ammonia is absorbed through rumen wall and converted to urea in the liver, which is either excreted through urine or recycled back to rumen. In this study, the values of ruminal-NH_3 contribution to suspended bacterial nitrogen fraction in groups 1 to 8 were: 42.85, 38.41, 38.62, 33.75, 49.81, 50.62, 47.24 and 49.42 per cent respectively. The plasma urea-N derived values from ruminal ammonia were: 35.33, 39.17, 39.45, 38.40, 32.20, 30.25, 29.40 and 35.85 per cent respectively in groups 1-8. The urinary-N derived from ruminal ammonia were: 34.15, 38.20, 20.42, 25.44, 20.57, 13.85, 16.41 and 19.80 per cent respectively in groups 1-8.

The data indicated that contribution of ruminal ammonia-N towards bacterial-N synthesis was more in OP fed groups as compared to HP fed groups in both the species and irrespective of aflatoxin intake. This indicated a better utilization of N by rumen microbes, when less amount of N was available whereas excess seemed to be either absorbed or it flowed more with ingesta into the lower part of digestive tract (Mathison and Milligan, 1971; Kennedy and Milligan, 1978a). The latter workers

also observed that the proportion of bacterial-N derived from $^{15}NH_3$ was considerably less when rumen ammonia pool was higher as compared to the proportion of bacterial-N derived from ruminal $^{15}NH_3$ in sheep fed protein deficient diets. Pilgrim et al. (1970) reported on an average 77 per cent of ^{15}N-incorporation of NH_3 from ^{15}N-ammonium sulphate into bacterial-N, when fed 12.5 g N/d as compared to 63 per cent incorporation given 22.2 g N/d in sheep. Dhiman and Arora (1985) observed earlier a similar trend in cattle and buffalo given high and low levels of nitrogen in the diet. They reported that the incorporation of $^{15}NH_3$ into bacterial-N was 42.95 and 51.52 per cent in calves fed optimum and sub-optimum diets, while the incorporation was 41.31 and 47.19 per cent in buffalo calves kept under feeding conditions similar to calves.

Plasma urea-N derived from ruminal-NH_3 indicated that higher amounts of urea-N were derived from ruminal-NH_3 in animals fed HP diet as compared to OP groups in calves of both the species, without any aflatoxin effect. The reason might be as a result of more absorption of ammonia through rumen wall in HP groups and greater ammonia release beyond the capacity of microbes to utilize it. Earlier workers (Egan and Kellaway, 1971; Kennedy and Milligan, 1978a, 1978b) observed that contribution of ruminal-NH_3 to plasma urea-N appeared to vary with the diet, especially with its protein content.

Superiority of buffalo calves over calves, showing more positive N balances and better utilization of absorbed N was evident

from the findings that urinary-N derived from ammonia-N was more in calves as compared to buffalo calves as earlier reported from this laboratory (Dhiman and Arora, 1985).

PHASE II

6.2.5 Effect of aflatoxin on digestibility of nutrients

Effect of aflatoxin on digestibility of nutrients in calves and buffalo calves fed OP and LP levels in the diet is given in Table 39. The digestibility of all the nutrients appeared to be less in LP groups as compared to OP fed animals, irrespective of species and aflatoxin intake.

6.2.6 Effect of aflatoxin on nitrogen balance

The data on nitrogen balance as a result of aflatoxin feeding given OP (groups 1-4) and LP (groups 5-8) levels in the diet are presented in Table 40. The total N intake in 8 groups was: 130.11, 123.02, 132.80, 129.10, 61.72, 62.93, 62.30 and 63.04 respectively. The average N intake in OP groups and LP groups was 128.76 and 62.49 g/day indicating that groups 5-8 were provided 51.47 per cent less nitrogen as compared to OP groups. The nitrogen (g/day) excreted in faeces was: Group 1: 45.63, Group 2: 43.75, Group 3: 50.0, Group 4: 44.38, Group 5: 37.50, Group 6: 36.89, Group 7: 34.37 and Group 8: 33.0. These results indicated that more N was excreted in faeces of animals given OP as compared to LP. But when the result were calculated as percentage of the intake excreted through faeces, the values were: Group 1: 35.07, Group 2: 35.56, Group 3: 37.65, Group 4: 34.38, Group 5: 60.76, Group 6: 53.62, Group 7: 55.16 and Group 8: 52.35, which indicated that more N was excreted through faeces in animals having low N intake.

Table 39. Effect of aflatoxin on digestibility coefficient of nutrients fed OP and LP diets (Phase II)

Group	Attribute					
	DM	OM	CP	CF	EE	NFE
1	61.99	65.21	64.92	55.27	79.90	69.14
2	63.80	67.29	64.44	58.67	77.19	70.75
3	62.13	65.32	62.35	57.57	74.05	68.58
4	59.30	62.90	65.62	55.63	69.17	65.39
5	50.74	[illegible]	39.24	50.16	55.77	61.58
6	52.50	[illegible]	41.38	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	44.83	51.85	68.80	61.19
8	[illegible]	[illegible]	[illegible]	[illegible]	70.09	62.29

These observations concurred with the results obtained in Phase I of this study and earlier work (Dhiman and Arora, 1985). Results also indicated that excretion of N through faeces was not affected by aflatoxin feeding in either of the species.

The quantity of N excreted (g/d) through urine and its percentage of N intake excreted through urine were: Group 1: 59.63, 45.83; Group 2: 65.0, 52.84; Group 3: 44.50, 33.51; Group 4: 51.06, 39.55; Group 5: 23.00, 37.27; Group 6: 25.56, 40.76; Group 7: 21.50, 34.50 and Group 8: 27.25, 43.22. These results indicated that excretion of N through urine was more in animals fed optimum amount of P as compared to low nitrogen fed animals. Results also indicated that excretion of N through urine was more in calves of both the species, fed aflatoxin, irrespective of protein levels.

Nitrogen retained (g/d) was: Group 1: 24.85, Group 2: 14.27; Group 3: 38.30, Group 4: 33.66, Group 5: 1.22; Group 6: 0.48, Group 7: 6.43 and Group 8: 2.79. The results indicated that all the animals were in positive nitrogen balance, but the nitrogen retention was more in OP fed animals as compared to LP fed groups. Nitrogen balances were less in calves of both the species fed aflatoxin, irrespective of the protein levels. Results also indicated that N balances (g/d) were more in buffalo calves as compared to crossbred calves, indicating better utilization in buffalo calves.

The amount of N retained as percent of absorbed N as such and when calculated on the basis of per kg live

Table 40. Effect of feeding aflatoxin on nitrogen balance fed OP and LP diets (Phase II)

Attribute	GROUP							
	1	2	3	4	5	6	7	8
Live weight (kg)	350.0	390.0	375.0	350.0	340.0	370.0	380.0	375.0
Total N intake (g/d)	130.11	123.02	132.80	129.10	61.72	62.93	62.30	63.04
Faecal N (g/d)	45.63	43.75	50.00	44.38	37.50	36.89	34.37	33.00
N absorbed (g/d)	84.48	79.27	82.80	84.72	24.22	26.04	27.93	30.04
N excreted in urine (g/d)	59.63	65.00	44.50	51.06	23.00	25.56	21.50	27.25
N retained (g/d)	24.85	14.27	38.30	33.66	1.22	0.48	6.43	2.79
N retained as percent of absorbed	29.41	18.00	46.26	39.73	5.04	1.84	23.02	9.28
N retained as percent of absorbed per kg live weight	0.084	0.046	0.123	0.114	0.015	0.005	0.061	0.025
N digestibility (%)	64.92	64.44	62.35	65.62	39.24	41.38	44.83	47.65
N retained as percent of intake	19.10	11.60	28.84	26.07	1.98	0.76	10.32	4.43
N excreted in faeces as percent of intake	35.07	35.56	37.65	34.38	60.76	58.62	55.16	52.35
N excreted in urine as percent of intake	45.83	52.84	33.51	39.55	37.27	40.76	34.50	43.22

weight were: Group 1: 29.41, 0.084; Group 2: 18.00, 0.046; Group 3: 46.26, 0.123; Group 4: 39.73, 0.114; Group 5: 5.04, 0.015; Group 6: 1.84, 0.005; Group 7: 23.02, 0.061 and Group 8: 9.28, 0.025 g/day. The data indicated that the values of these parameters were more in animals fed OP than LP fed groups and buffalo calves appeared to be superior to crossbred calves, when examined on the basis of these parameters. Aflatoxin fed animals given either level of protein retained less N as percent of absorbed N as well as when it was calculated on the basis of per kg live weight.

6.2.7 Effect of aflatoxin on ruminal NH_3-N concentration, water and ammonia outflow from the rumen fed OP and LP diets

The ruminal ammonia-N concentration at different hours is given in Table 41. The mean ruminal ammonia-N concentration (mg/100 ml SRL) in different groups was: Group 1: 13.72 ± 2.44, Group 2: 12.68 ± 1.12, Group 3: 14.0 ± 1.96, Group 4: 14.20 ± 2.33, Group 5: 6.16 ± 0.51, Group 6: 7.20 ± 0.71, Group 7: 5.52 ± 0.37 and Group 8: 6.22 ± 0.66. These results indicated that the mean ruminal-NH_3-N concentration irrespective of aflatoxin treatment was higher in OP fed groups as compared to LP groups.

Estimates of rumen fluid volume and water flow rates to the lower digestive tract, determined with the help of ^{51}Cr-EDTA using isotopic dilution technique are presented in Table 42. The flow of ammonia-N (g/d) to the lower digestive tract was also calculated as described

Table 41. Effect of feeding aflatoxin on ruminal NH -N (mg/100 ml SRL) concentration fed OP and LP diets

Group	Hours after isotope injection										Average ± S.E.
	0	1	2	3	5	7	9	12	18	24	
1	7.20	12.40	20.80	25.60	26.00	14.40	8.40	6.40	6.80	9.20	13.72 ± 2.44
2	8.00	10.00	12.00	14.00	19.20	16.00	14.40	14.00	11.20	8.00	12.68 ± 1.12
3	10.00	10.40	15.20	18.40	24.00	21.20	18.00	9.60	6.60	6.40	14.00 ± 1.96
4	8.80	15.20	21.60	22.00	23.60	21.60	9.60	8.00	5.60	6.00	14.20 ± 2.33
5	4.80	6.60	7.20	8.40	9.00	5.60	5.60	5.60	4.80	4.00	6.16 ± 0.51
6	6.80	7.60	8.40	8.80	11.60	6.80	6.80	6.40	6.00	2.80	7.20 ± 0.71
7	4.20	6.60	7.00	7.80	5.00	5.00	5.00	5.00	5.00	4.60	5.52 ± 0.37
8	6.00	6.00	9.00	9.00	9.00	5.20	4.50	3.50	6.00	4.00	6.22 ± 0.66

Table 42. Effect of aflatoxin intake on water and ammonia flow from rumen fed OP and LP diets (Phase II)

Group	Body weight (kg)	Ruminal NH_3-N concentration (mg/l)	Rumen volume (l)	Rumen fluid outflow rate (l/d)	Ammonia outflow with rumen fluid (g N/d)*	Rumen volume (l) % body wt.
1	350.0	13.72	39.50	85.40	11.72	11.28
2	390.0	12.68	57.45	95.20	12.06	14.73
3	375.0	14.00	48.50	101.40	14.20	12.93
4	350.0	14.20	56.40	120.60	17.13	16.12
5	340.0	6.16	45.50	92.50	5.70	13.38
6	370.0	7.20	55.20	90.00	6.48	14.92
7	380.0	5.72	51.00	91.50	5.23	13.42
8	375.0	6.22	43.50	110.25	6.86	11.60

* Calculated as the product of outflow (l/d) and measured mean NH_3-N concentration (g/l) in rumen fluid

in Phase I (6.2.3). The rumen volume (l) in different groups was: Group 1: 39.50, Group 2: 57.45, Group 3: 48.50, Group 4: 56.40, Group 5: 45.50, Group 6: 55.20, Group 7: 51.00 and Group 8: 43.50. Results indicated that rumen volumes were not affected due to aflatoxin intake, species difference or level of protein. Rumen volume as percent of body weight in different groups was: Group 1: 11.28, Group 2: 14.73, Group 3: 12.93, Group 4: 16.12, Group 5: 13.38, Group 6: 14.92, Group 7: 13.42 and Group 8: 11.60. It was apparent from these data that rumen volume as percent of body weight varied from 11.28 to 14.92 in crossbred calves and 11.60 to 16.12 in buffalo calves. These values are similar to the values obtained in Phase I of this experiment.

The ammonia-N outflow from rumen fluid was much more in animals fed OP diet as compared to LP fed groups and the values in different groups were: Group 1: 11.72, Group 2: 12.06, Group 3: 14.20, Group 4: 17.13, Group 5: 5.70, Group 6: 6.48, Group 7: 5.23 and Group 8: 6.86

6.2.8 Effect of aflatoxin on the kinetics of ammonia in rumen and blood fed OP and LP diets

The changes at different time intervals in ^{15}N enrichment of NH_3-N in the rumen fluid and enrichment of rumen bacterial-N, plasma urea-N and urinary-N after single intra-ruminal injection of ^{15}N-ammonium sulphate fed OP and LP diets with or without aflatoxin are depicted in Figs. 23 to 30 and the data is presented in Table 43.

FIG. 23. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

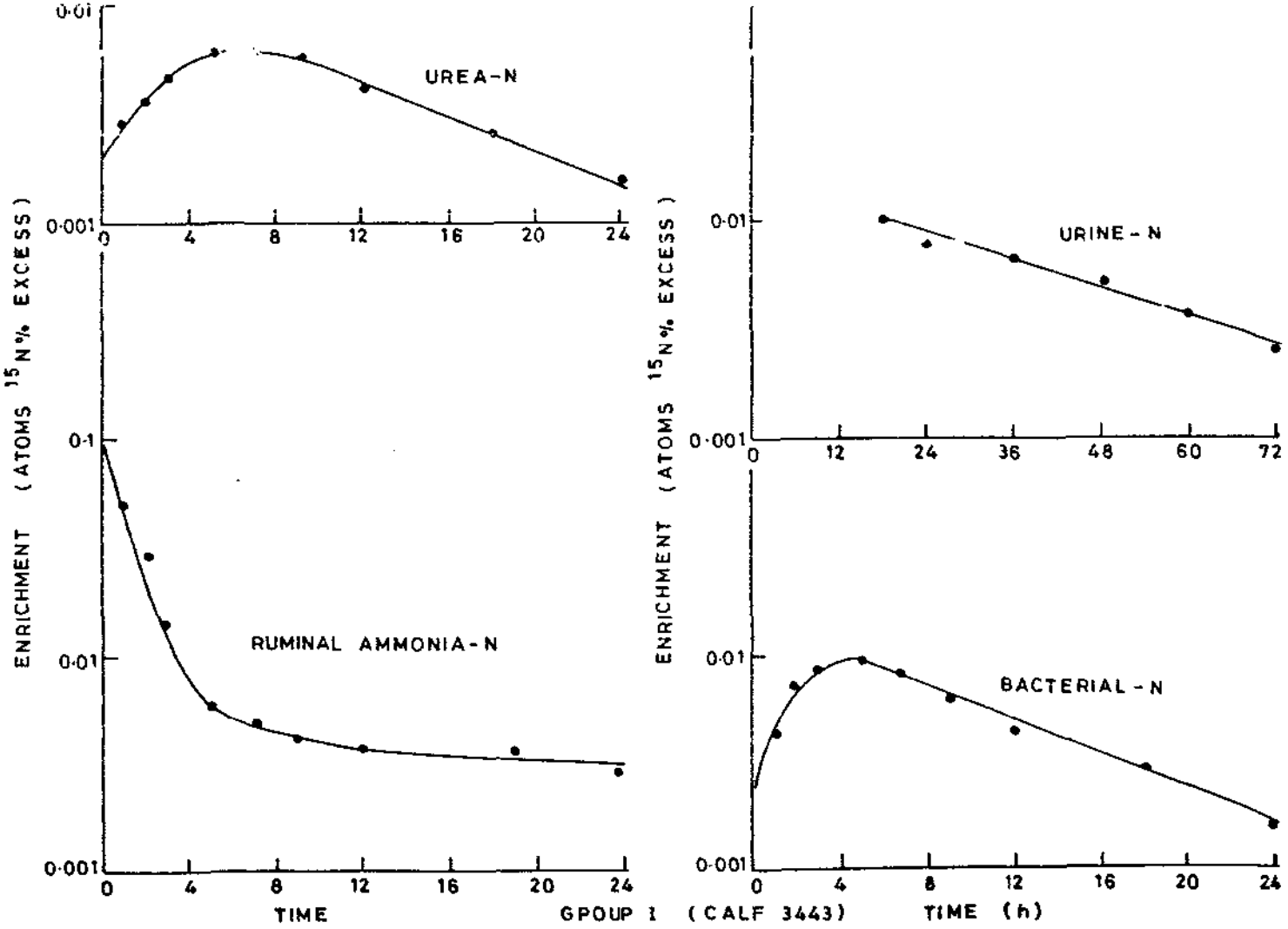


FIG. 24. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

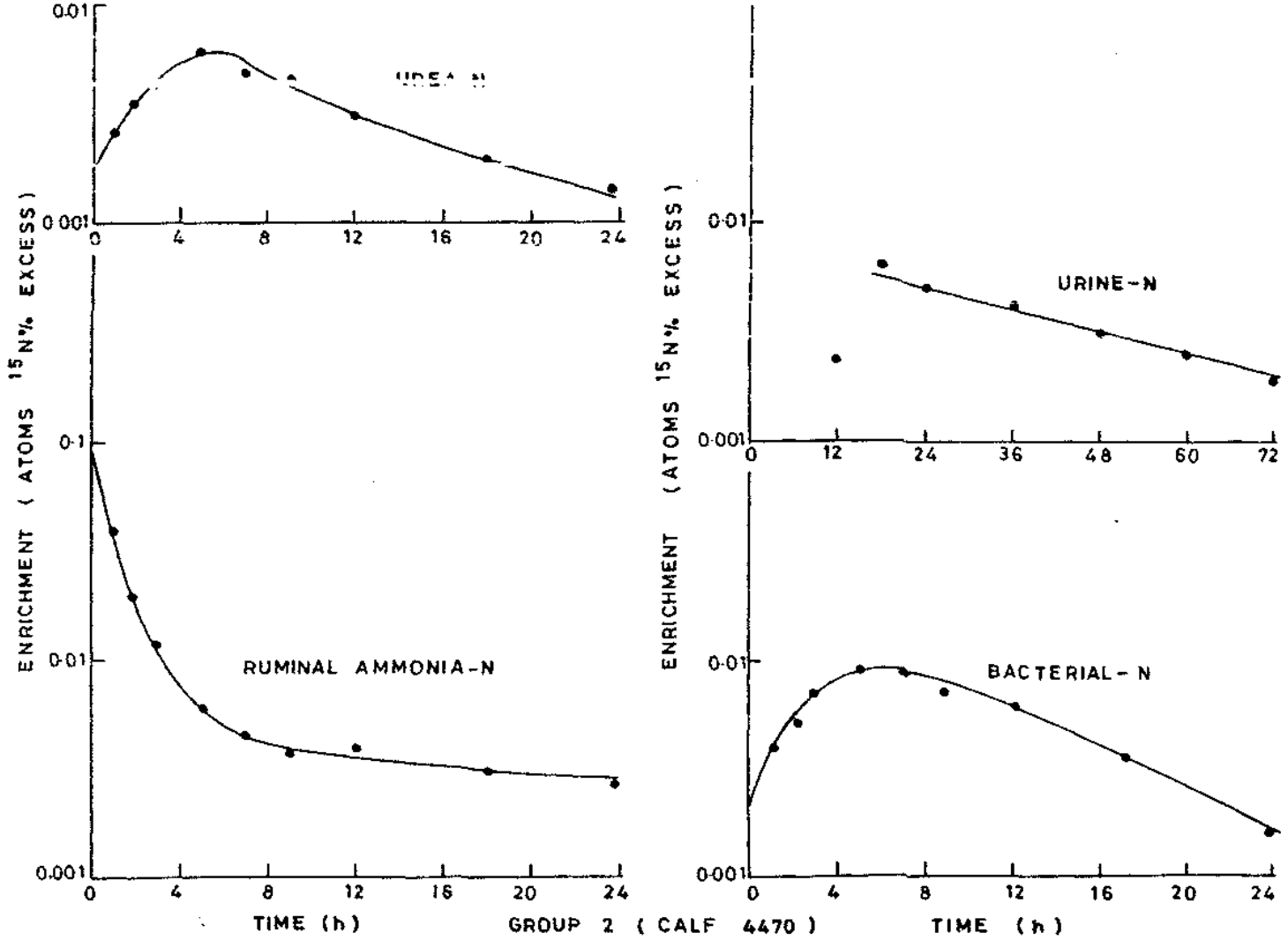


FIG. 25. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

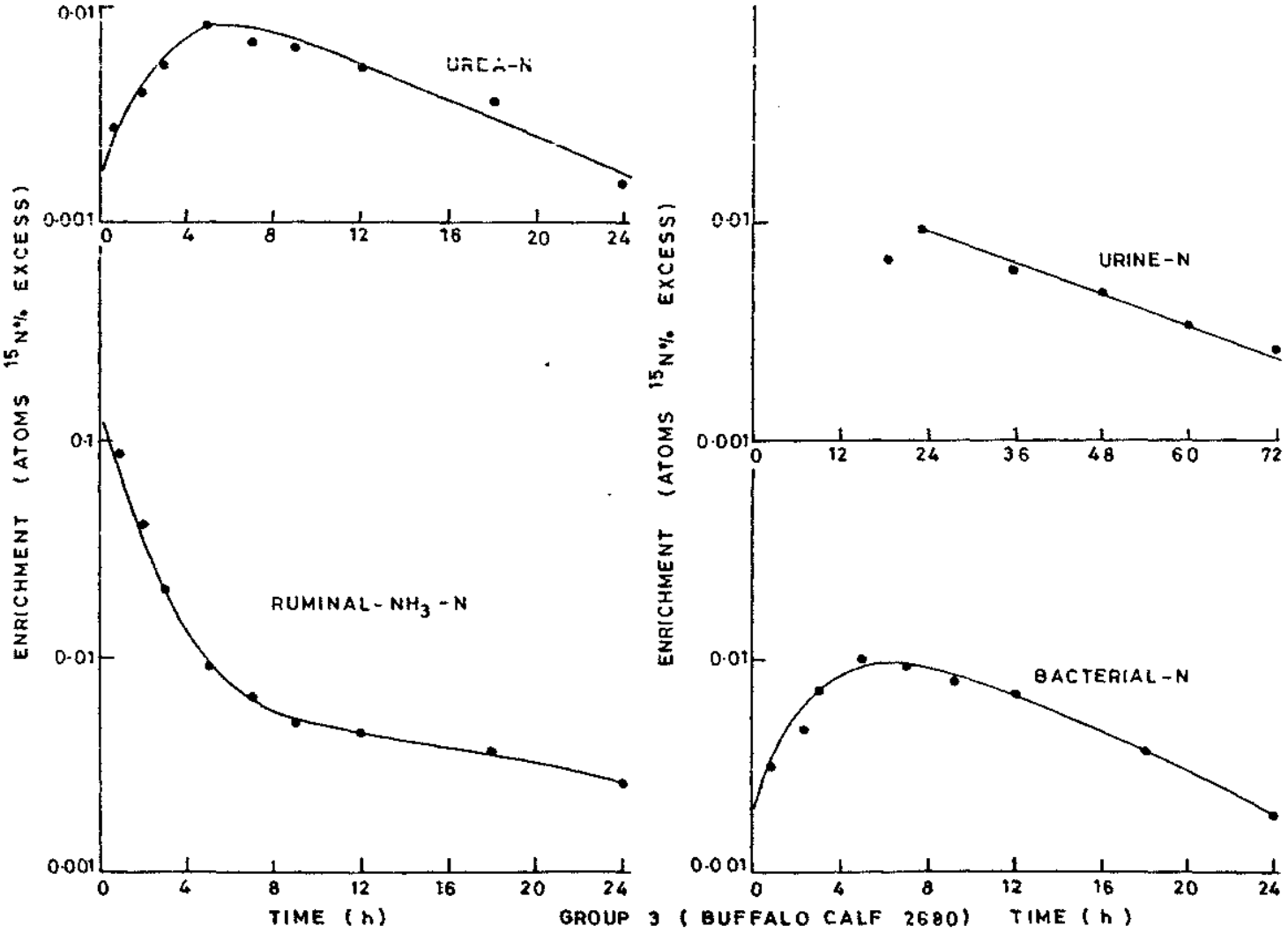


FIG. 26. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

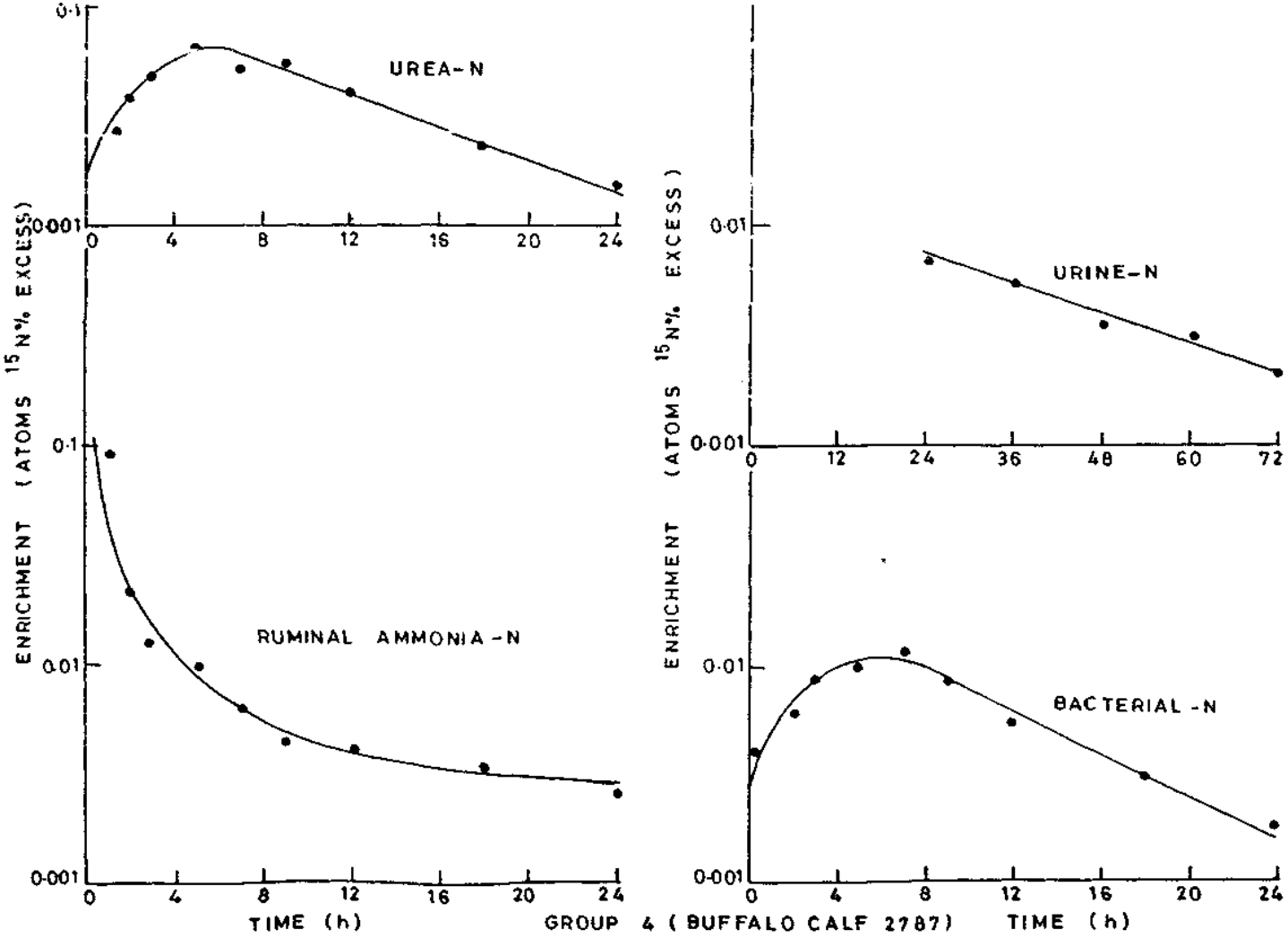


FIG. 27. ENRICHMENT OF RUMINAL AMMONIA-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

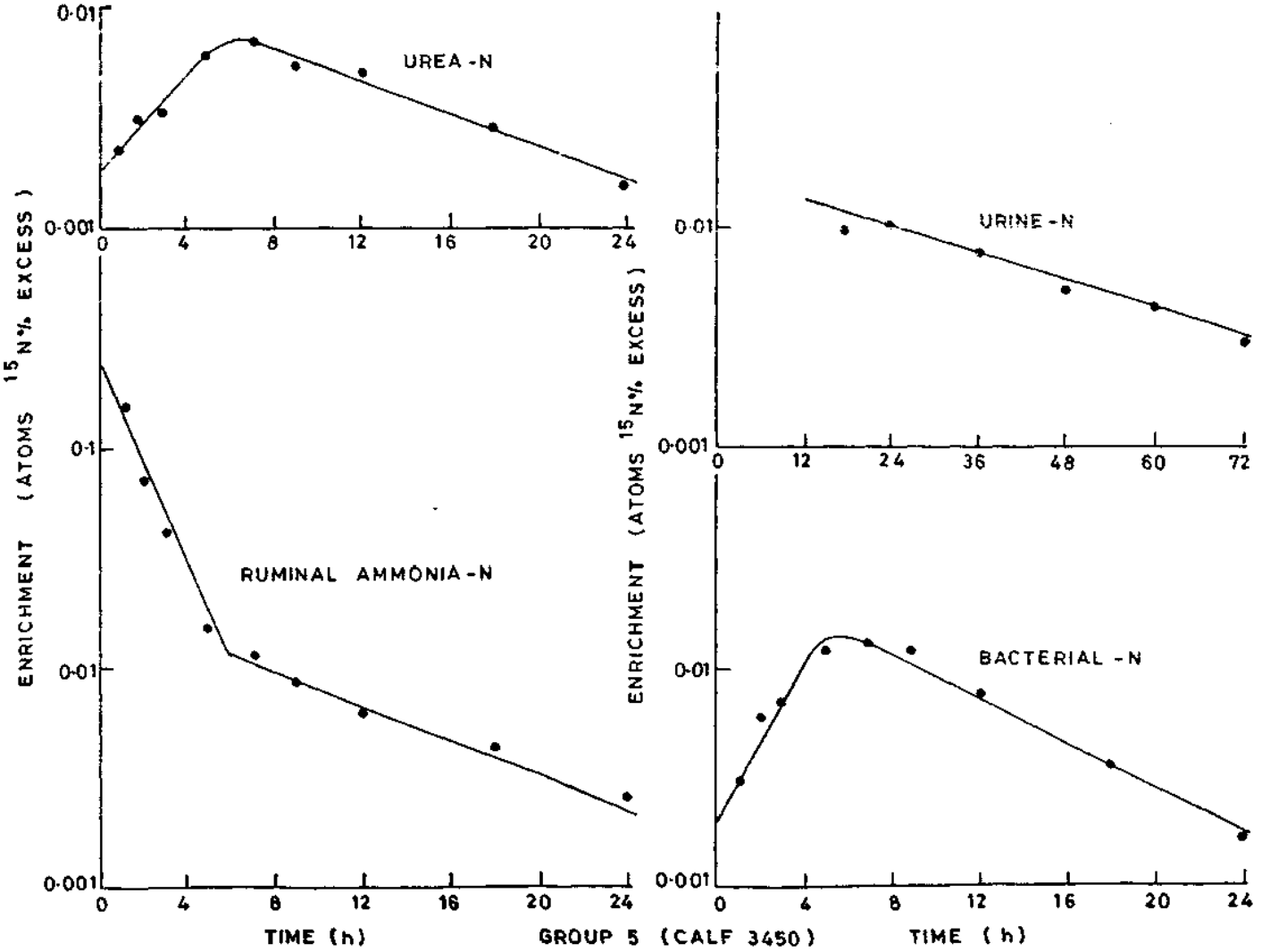


FIG.28. ENRICHMENT OF RUMINAL AMMONIA-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

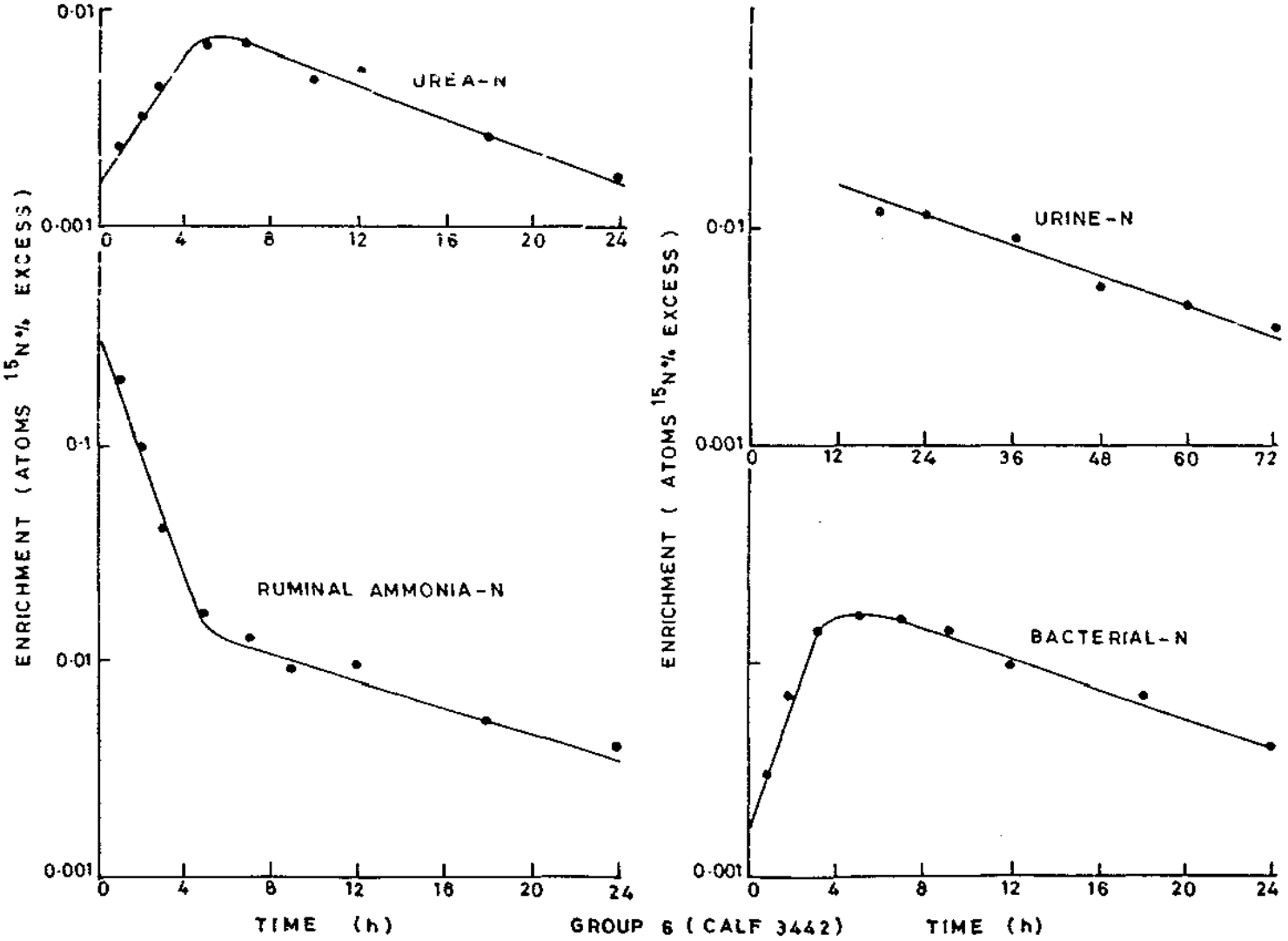


FIG.29. ENRICHMENT OF RUMINAL NH_3-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

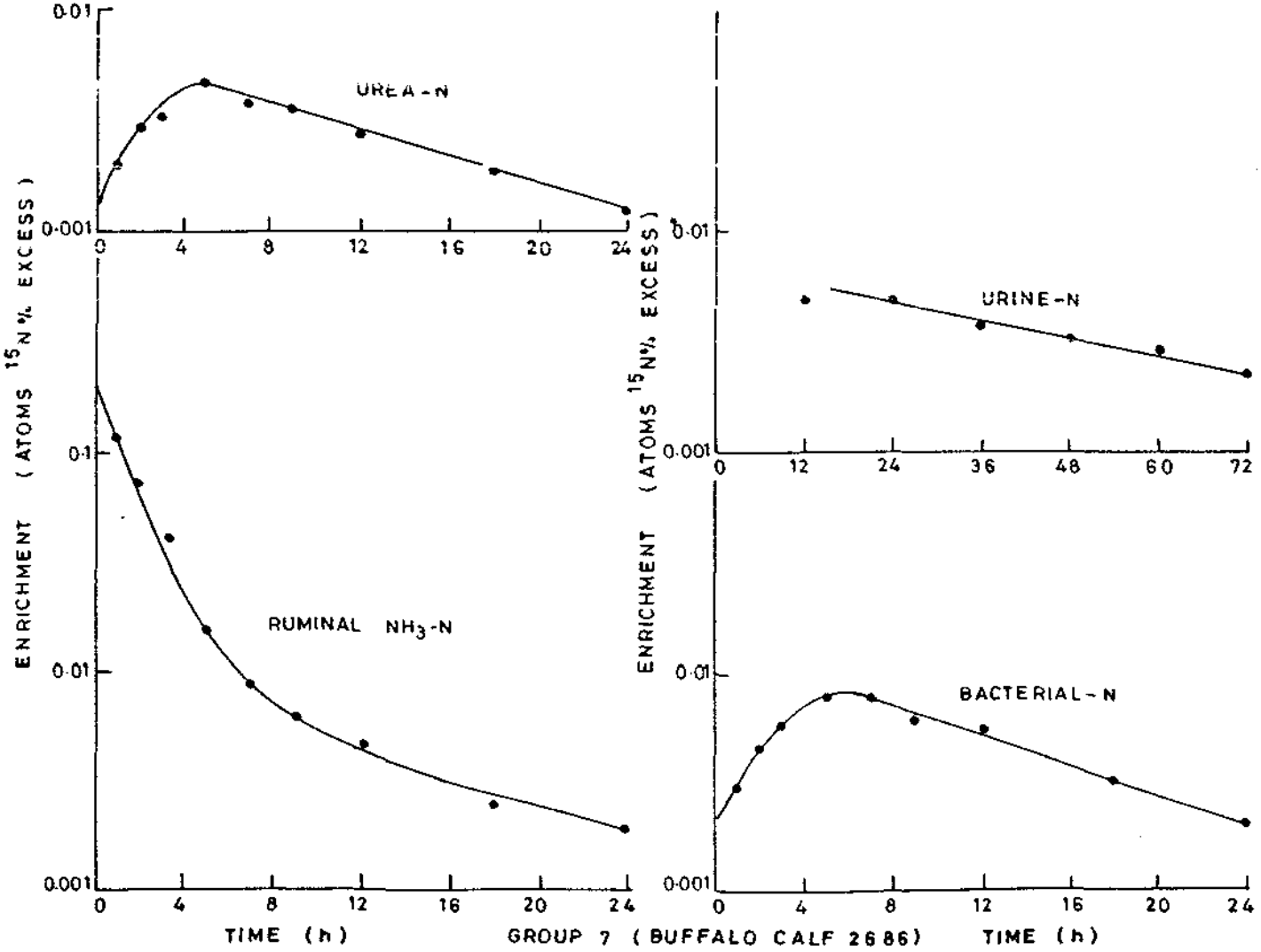


FIG .30. ENRICHMENT OF RUMINAL AMMONIA-N, BACTERIAL-N, PLASMA UREA-N AND URINE-N WITH TIME AFTER A SINGLE INTRA-RUMINAL INJECTION OF (^{15}N) AMMONIUM SULPHATE

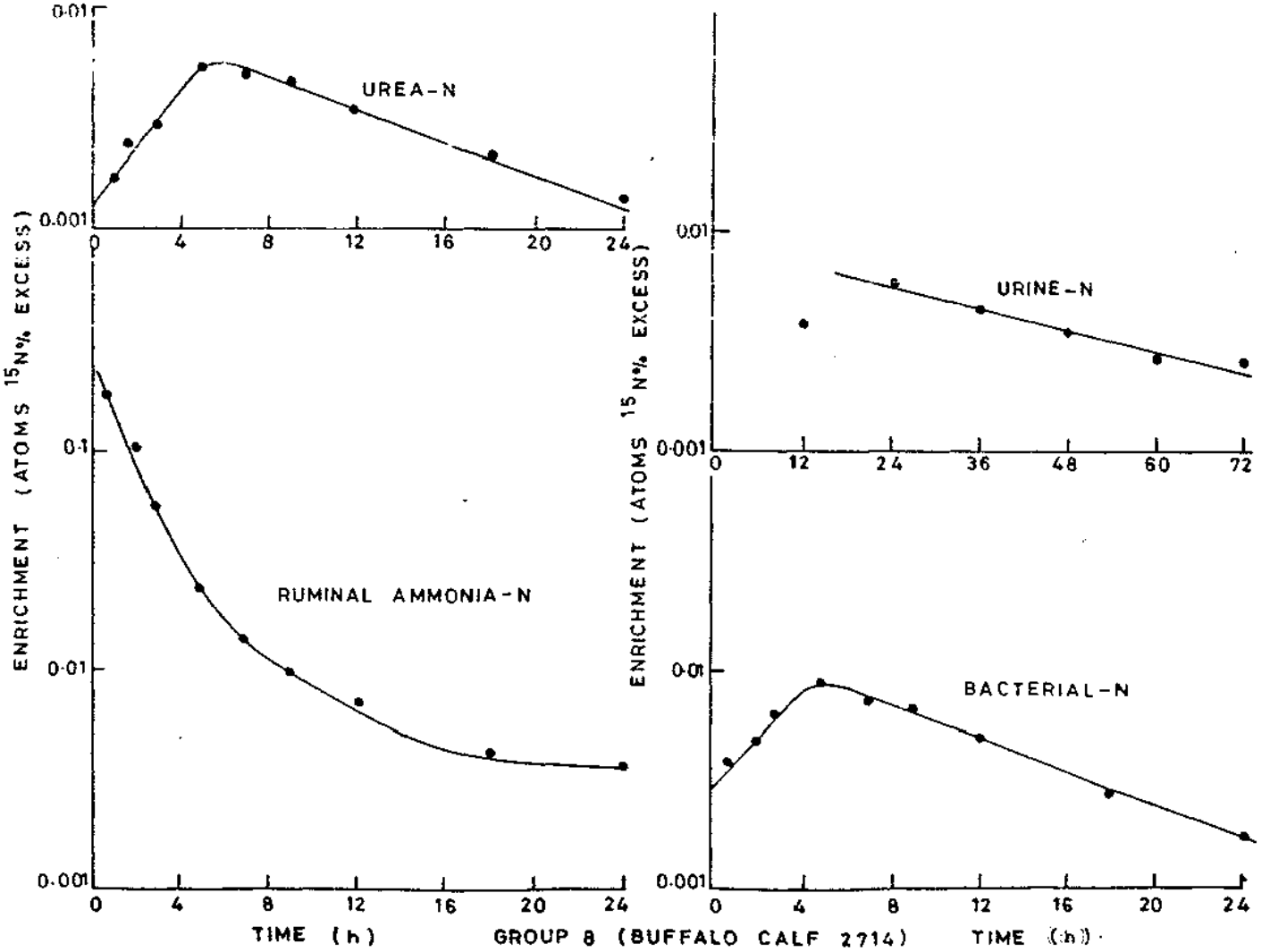


Table 43. Kinetics of ammonia in rumen and blood given aflatoxin with two levels of protein using single injection of ^{15}N-ammonium sulphate (Phase II)

Group	Rumen NH_3 pool size (g N)	Ruminal NH_3 entry rate (g N/d)	Ruminal NH_3 irreversible loss rate (g N/d)	NH_3 irreversible loss rate as (%) of entry rate	Recycling rate (g N/d)	Suspended bacterial-N derived from ruminal ammonia (%)	Plasma urea-N derived from ruminal ammonia (%)	Urinary N derived from ruminal ammonia (%)
1	3.30	25.87	14.48	55.97	11.39	52.64	36.38	24.35
2	2.85	24.07	18.06	75.03	6.01	51.50	39.46	28.20
3	3.12	21.05	12.95	61.52	8.10	53.60	32.56	18.20
4	2.90	19.65	12.86	65.44	6.79	49.54	33.48	24.45
5	1.94	15.26	9.87	64.68	5.39	61.75	25.15	20.75
6	1.81	13.45	9.35	69.52	4.10	65.87	22.28	25.78
7	2.16	15.44	9.56	61.92	5.88	70.76	18.20	19.47
8	2.23	16.57	11.05	66.08	5.52	71.87	19.26	28.32

The ruminal-NH_3 pool sizes in different groups were: Group 1: 3.30, Group 2: 2.85,Group 3: 3.12, Group 4: 2.90, Group 5: 1.94, Group 6: 1.81, Group 7: 2.16 and Group 8: 2.23, indicating that size of ammonia pool was more in animals given OP as compared to LP fed animals irrespective of species and aflatoxin intake.

Ruminal-NH_3 entry rates were 25.87, 24.07, 21.05, 19.65, 15.26, 13.45, 15.44 and 16.57 g N/d respectively in 8 groups. Higher ammonia entry rates in groups 1 to 4 seemed to be again due to more nitrogen intake as compared to other 4 groups. Species difference and aflatoxin dose level had no effect on rumen ammonia entry rates.

The irreversible loss rates of ammonia-N (g N/d) as such and as a percent of ammonia entry rates in different groups were: Group 1: 14.48, 55.97; Group 2: 18.06, 75.03; Group 3: 12.95, 61.52; Group 4: 12.85, 65.44; Group 5: 9.87, 64.68; Group 6: 9.35, 69.52; Group 7: 9.56, 61.92 and Group 8: 11.05, 66.68. These results showed higher irreversible loss of ammonia-N in groups fed aflatoxin, irrespective of species and level of protein.

The rates of recycling of ammonia to the rumen NH_3 pool were 11.39, 6.01, 8.10, 6.79, 5.39, 4.10, 5.88 and 5.52 g N/d in groups 1 to 8 respectively. Recycling rates were more in animals given more nitrogen,irrespective of species and aflatoxin intake.

The contribution of ruminal ammonia to different nitrogen fractions in groups 1 to 8 were: 52.64, 51.50,

53.60, 49.54, 61.75, 65.87, 70.76 and 71.87 per cent respectively for bacterial-N; 36.38, 39.46, 32.56, 33.48, 25.15, 22.28, 18.20 and 19.26 per cent respectively for plasma urea-N; 24.35, 28.20, 18.20, 24.45, 20.75, 25.78, 19.47 and 28.32 per cent respectively for urinary-N.

According to the results mentioned above, the contribution of ruminal ammonia-N towards suspended bacterial-N was more in calves and buffalo calves fed LP diet as compared to OP fed animals, which indicated better utilization of ammonia-N under N scarcity conditions. This is further supported by the fact that plasma urea-N were less in LP fed groups. Aflatoxin did not seem to affect the rumen fermentation. Higher irreversible loss of ammonia-N as percent of total ammonia-N entry rate and more excretion of N through urine in aflatoxin fed calves and buffalo calves at all levels of protein indicated that aflatoxin affected the nitrogen metabolism at tissue levels.

Results obtained in both the phases indicated that there was no effect of feeding aflatoxin on digestibility of nutrients. Digestibility of nutrients was more in groups fed OP protein as compared to LP groups. Nitrogen balances (g/day) were positive in all the animals irrespective of level of protein and species. Nitrogen excretion through urine (g/day) was more in calves and buffalo calves fed aflatoxin. Buffalo calves seemed to be superior over calves showing more positive N balance and better utilization of absorbed N.

Results further showed that contribution of ruminal-NH_3 towards bacterial protein and plasma urea-N synthesis was inversely and directly related with the nitrogen intake, indicating better utilization of ruminal-NH_3 when less N was available. The contribution of ruminal-NH_3 towards urinary-N was more in animals fed HP than OI ; and OP fed animals had more contribution of ruminal NH_3 towards their urinary-N than LP fed groups. Aflatoxin effect was evident in the greater excretion pattern of ruminal-ammonia quantity through urinary-N which was recorded more in cattle than buffalo calves as a species difference.

CHAPTER 7

Experiment 5:

Tissue distribution and excretion pattern of aflatoxin in lactating goats

TISSUE DISTRIBUTION AND EXCRETION PATTERN OF AFLATOXIN IN LACTATING GOATS

The present study was undertaken to examine the effect of feeding aflatoxin on digestibility of nutrients, excretion and tissue distribution of radioactive aflatoxin in lactating goats.

7.1 MATERIALS AND METHODS

7.1.1 Plan of the experiment:

Twelve Alpine, Beetal and Saaren crosses in their early lactation were selected from the herd of National Dairy Research Institute, Karnal. They were randomly divided into two groups of six goats each. All of them were fed concentrate mixture and green maize as per their requirements (NRC, 1981). Group 1 served as a control, while the goats in group 2 were given aflatoxin @ 1.0 ppm of their DM intake and was called as experimental group. All the goats were milked twice daily at 6.00 A.M. and 4.00 P.M. respectively. After adapting the animals on this feeding regimen for 35 days, a metabolism trial was conducted to know the digestibility of nutrients. After the metabolism trial was over, 3 goats from experimental group were given an oral dose of ^{3}H-AFB_1 and samples of milk, urine and faeces were collected at different time intervals upto 24 h. After 24 h, these goats were sacrificed and various tissues were collected to know the tissue distribution of its radioactivity. The particulars of goats and their distribution in two groups are presented in Table 44.

Table 44. Particulars of lactating goats and their distribution

Treatment group	Goat No.	Body weight (kg)	Lactation No.	Milk yield (kg)
Group 1 (0 ppm Af) Control	SB 268	40.0	3	1.6
	ASB 355	40.5	2	1.9
	ASB 361	34.5	2	1.1
	AB 609	38.5	2	1.3
	AB 672	35.2	1	1.2
	AB 680	36.5	1	1.5
Mean		37.53		1.27
Group 2 (1.0 ppm Af)	SB 291	42.0	2	1.2
	ASB 369	39.0	2	1.5
	ASB 381*	39.0	2	1.6
	ASB 392	32.8	2	1.3
	AB 657*	39.5	2	1.7
	AB 677*	32.0	1	1.5
Mean		37.38		1.47

* Goats used for ^{3}H-APB_1 excretion and tissue distribution studies

7.1.2 Housing and management of goats:

Goats were kept individually in pens of size 1.5 m x 2.0 m. The pens were thoroughly white washed and disinfected before the start of the experiment. Disinfection was carried out daily in the morning at the time of cleaning of the pens.

7.1.3 Feeding schedule and milking of the goats:

Goats in both the groups were fed on concentrate mixture and green maize as per their requirements (NRC, 1981). The concentrate mixture was constituted of 32 parts groundnut cake, 40 parts maize, 25 parts wheat bran, 2 parts mineral mixture and 1 part common salt. In addition, goats in group 2 were given an oral dose of aflatoxin @ 1.0 ppm of their DM intake. Water was offered ad libitum twice daily, i.e., at 9.00 A.M. and 3.30 P.M. throughout this study. All the goats were milked twice daily, i.e. at 6.00 A.M. and 4.00 P.M. respectively. The chemical composition of concentrate mixture and green maize is presented in Table 45.

Table 45. Chemical composition of feed(%DM basis)

Feed	CP	EE	CF	NFE	Ash
Concentrate mixture	24.19	3.67	4.58	60.36	7.20
Green maize	9.69	1.71	22.00	57.38	9.22

7.1.4 Studies on the digestibility of nutrients:

After a preliminary feeding of 35 days, all the 12

lactating goats were shifted to metabolic crates, having an arrangement of separate collection of faeces and urine. They were given their concentrate mixture quota at 8.00 A.M. and green maize was offered at 10.00 A.M. daily. Water was offered ad libitum twice daily.

A metabolism trial of 7 days was conducted to know the digestibility of nutrients and nitrogen balance. During the metabolism trial, samples of feed, faeces, residue, urine and milk were collected and stored for the analysis of proximate principles as per AOAC (1975).

7.1.5 Studies on excretion pattern and tissue distribution of aflatoxin:

7.1.5.1 Administration of ^{3}H-AFB_1:

After the metabolism trial was over, 3 goats from group 2, particulars given in Table 41, were selected for studying the tissue distribution and excretion pattern of aflatoxin B_1. Each goat was administered orally 200 µCi of ^{3}H-AFB_1, procured from M/s. Moravek Biochem. Inc., Mercury Lane, U.S.A., along with 3 g of maize flour.

7.1.5.2 Sample collection:

The goats were housed in metabolism cages for 24 h after administration of ^{3}H-AFB_1. Samples of urine were collected at 0 (pre-dosing), 3, 6, 12, 18 and 24 h post-dosing while faeces and milk samples were collected at an interval of 6 h upto 24 h. These samples were stored frozen till analysis.

After 24 h, these goats were sacrificed by injecting

50 ml of saturated solution of $MgSO_4$ through jugular vein. Tissues were removed, weighed and stored under frozen conditions till analysed.

7.1.5.3 Assay of radioactivity:

Aliquots of urine were added directly to dioxane based scintillation fluid (Bray, 1960) having the following composition:

Napthalene	=	60 g
PPO	=	4 g
POPOP	=	0.2 g
Methanol (absolute)	=	100 ml
Ethylene glycol	=	20 ml

P-dioxane to make 1 l

Radioactivity was measured in a Packard Liquid Scintillation Counter (Model Prias BPLD)

Faeces and tissue samples were homogenized with water and 1 ml of homogenate (having about 100 mg solid sample) was transferred to scintillation vial (Wogan et al., 1967). Further processing of the samples was done according to Mabee and Chiple (1973). One ml soluene (tissue dissolver obtained from M/s. Packard Instrument Company, USA) was added to each vial and the vials were incubated in a water-bath until dissolution was complete. After incubation, 0.01 ml of 30 per cent hydrogen peroxide was added to each vial to decolorize the samples. All the vials were shaken and 0.01 ml of concentrated HCl was added to each vial to neutralize pH. The vials were permitted to stand 15 min at room temperature and 5 ml of the liquid scintillation counting solution was added to each vial. The samples were

counted for radioactivity as described earlier. Milk samples were lyophilized and 50 mg lyophilized sample was transferred to scintillation vial and processed and counted for radioactivity as per Mabee and Chipley (1973).

7.1.6 Statistical analysis of the data:

The data were analysed as per Snedecor and Cochran (1967).

7.2 RESULTS AND DISCUSSION

7.2.1 Effect of feeding aflatoxins on digestibility of nutrients:

The dry matter intake and digestibility of nutrients in two groups are presented in Table 46. The digestibility coefficients of nutrients were: DM 73.1 ± 1.58, 73.95 ± 1.22; OM 75.30 ± 1.30, 76.60 ± 1.40; CP 74.64 ± 1.47, 77.38 ± 1.02; CF 68.62 ± 1.93, 68.06 ± 1.40; EE 79.67 ± 3.90,77.38 ± 3.66 and NFE 77.96 ± 0.97, 78.70 ± 1.66 in two groups respectively. These results indicated that there was no effect of feeding of this level of aflatoxin on any proximate principle. Sinha (1983) also reported similar results in crossbred kids fed a diet with or without 1 ppm aflatoxin.

The dry matter intake was 1.22 ± 0.074 and 0.840 ± 0.072 kg/d respectively in the two groups. The difference in dry matter intake was significant ($P < 0.01$) when the results were compared on the basis of dry matter intake/kg $W^{0.75}$. Similar decrease in dry matter intake was observed by Lynch et al. (1971) in calves fed aflatoxin upto 0.10 mg/kg body weight for six weeks.

Table 46. Effect of aflatoxin on dry matter intake and digestibility coefficients of nutrients

Attribute	Group 1 (0 ppm Af) Control	Group 2 (1.0 ppm Af)
DM intake (kg/d)	1.22 ± 0.074*	0.840 ± 0.072*
Dry matter	73.1 ± 1.58	73.95 ± 1.22
Organic matter	75.30 ± 1.30	76.60 ± 1.40
Crude protein	74.64 ± 1.47	77.18 ± 1.02
Crude fibre	68.62 ± 1.93	68.06 ± 1.40
Ether extract	79.67 ± 3.90	77.18 ± 3.66
Nitrogen free extract	77.96 ± 0.97	78.70 ± 1.66

* $P < 0.05$

7.2.2 Effect of aflatoxin on nitrogen balance:

The data presented in Table 47 revealed that the nitrogen intake in groups 1 and 2 was 26.28 ± 1.94 and 18.32 ± 1.44 g/day, a significant less ($P < 0.01$) intake in group 2. The excretion of N through faeces was significantly more ($P < 0.05$) in experimental group as compared to control group but with no statistical significant difference in N excretion calculated as percentage of N intake.

The excretion of N through urine (g/day) was 7.83 ± 0.69 and 8.56 ± 0.76 in groups 1 and 2 respectively, which was not statistically different. But N excreted in urine as percent of total N intake was 30.13 ± 2.20 and 43.46 ± 2.53 indicating a significant effect ($P < 0.01$), similar to the observations of earlier workers (Lynch et al., 1971; Hale and Wilson, 1979).

There was no effect of aflatoxin on N excretion through milk as such or when calculated as a percent of N intake.

The amount of N retained was 7.63 ± 1.33 and 3.13 ± 0.69 g in groups 1 and 2 respectively. The results indicated that goats in both the groups were in positive N balance but the quantity of N balance was significantly higher ($P < 0.01$) in control group as compared to aflatoxin fed group. N retained as percent of absorbed N was 37.56 ± 4.57 and [illegible] indicating higher utilization in group 1 as compared to group 2. Similarly, N retained as percent of absorbed N per kg live weight was also significantly more in group 1 than group 2, the results supported by the findings

Table 47. Effect of aflatoxin on nitrogen balance

Attribute	Group 1 (0 ppm Af) Control	Group 2 (1.0 ppm Af)
Body weight (kg)	37.53 ± 1.03	37.38 ± 1.64
N intake (g/d)	**26.28 ± 1.94	**18.32 ± 1.44
N excreted in faeces (g/d)	* 6.59 ± C.47	* 4.16 ± 0.42
N absorbed (g/d)	*19.69 ± 1.66	*14.16 ± 1.09
N excreted in urine (g/d)	7.83 ± 0.69	8.56 ± 0.76
N excreted in milk (g/d)	4.23 ± 0.32	3.13 ± 0.35
N retained (g/d)	** 7.63 ± 1.33	** 3.13 ± 0.69
N retained as % of absorbed	*37.56 ± 4.57	*21.35 ± 3.60
N retained as % of absorbed per kg live wt	* 1.02 ± 0.35	* 0.59 ± 0.28
N digestibility (%)	74.64 ± 1.47	77.38 ± 1.02
N retained as % of intake	*28.33 ± 3.87	*16.48 ± 2.69
N excreted in faeces as % of intake	25.37 ± 1.47	22.62 ± 1.35
N excreted in urine as % of intake	**30.13 ± 2.20	**43.46 ± 2.53
N excreted in milk as % of intake	16.17 ± 0.63	17.44 ± 1.98

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

of earlier workers (Lynch et al., 1971; Hale and Wilson, 1979; Neathery et al., 1980).

7.2.3 Excretion pattern of ^{3}H-aflatoxin B_1:

7.2.3.1 Urine:

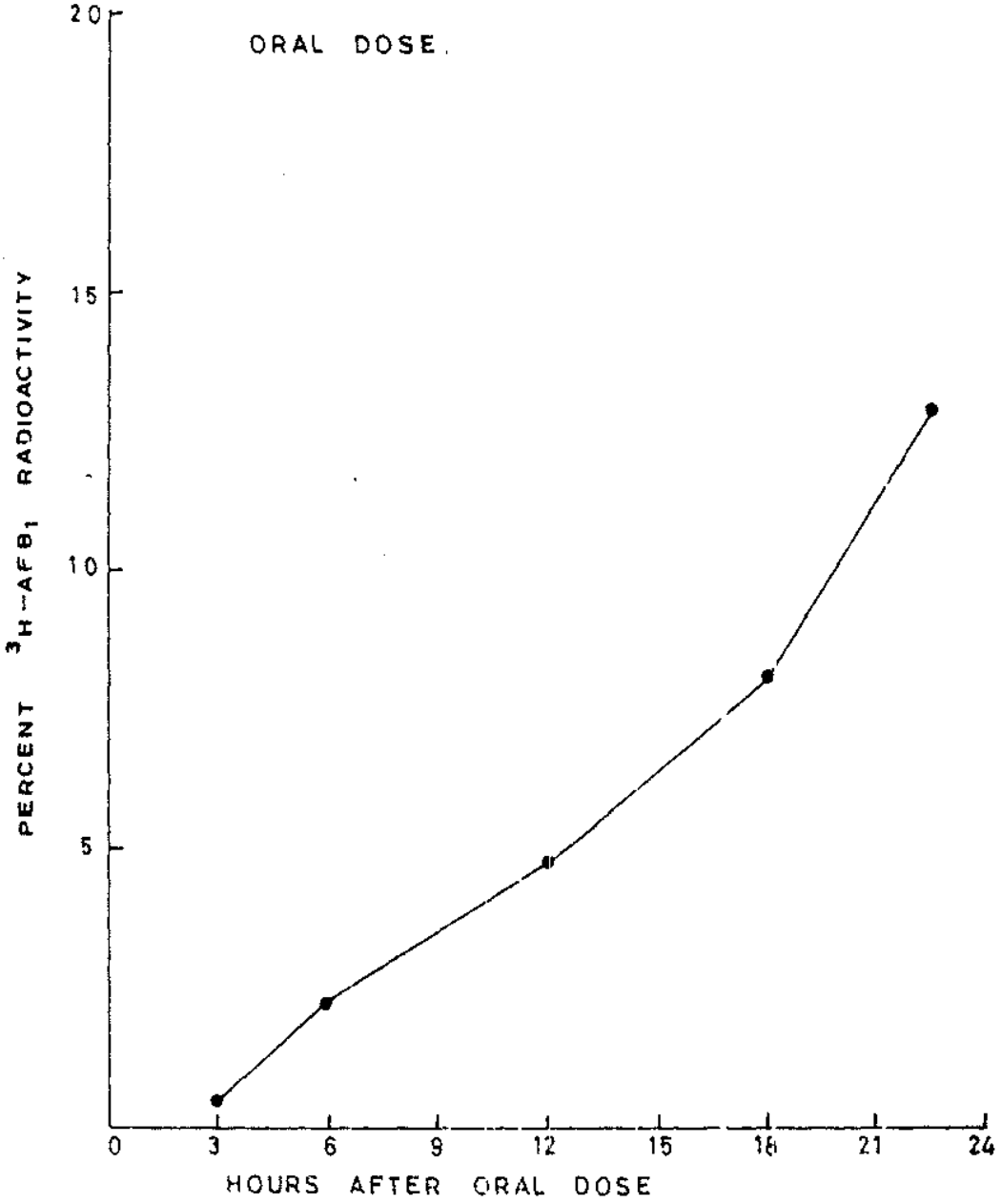
The percent radioactivity of the dose administered orally, excreted through urine in 3 goats is presented in Table 48 and depicted in Fig. 31. The radioactivity in urine was detected as early as 3 h post-dosing in all the three goats which indicated a rapid absorption of aflatoxin from the gastro-intestinal tract. Polan et al. (1974) and Helferich et al. (1986) also observed rapid absorption of ^{3}H or ^{14}C-AFB_1 given orally in cows and goats respectively. Polan et al. (1974) observed that blood tritium counts increased from 20 CPM/ml at 2 h to 40 CPM at 4 h, post-dosing of ^{3}H-AFB_1 in lactating cows. Helferich et al.(1986) reported that goats receiving ^{14}C-AFB_1 orally had detectable radioactivity in blood within 30 minutes.

The average cumulative excretion of radioactivity in 3 lactating goats was 12.93 ± 0.88 per cent of the radioactive dose administered orally. The excretion rate is quite high as compared to 3.4 per cent radioactivity excreted in 96 h in lactating cows (Polan et al., 1974). This might be due to the species difference or dose effect (Helferich et al., 1986). They also reported that urinary excretion in the lactating goat was greater than observed in rats. The cause of the species difference may be greater conjugation capacity of the O-demethylated metabolite of AFB_1, with soluble nucleophiles in lactating goat, which render

Table 48. Excretion of ^{3}H-AFB_1 through urine, faeces and milk

Time after dosing (h)	Goat No. 381	Goat No. 657	Goat No. 677	Average
	Percent of total dose administered			
URINE				
3	0.25	0.40	0.45	0.37
6	1.50	2.10	1.90	1.83
12	2.60	2.50	3.20	2.77
18	2.96	3.15	4.05	3.39
24	4.10	4.75	4.86	4.57
Total	11.41	12.90	14.46	12.93
FAECES				
6	2.00	1.90	2.20	2.03
12	5.10	6.20	5.80	5.70
18	4.50	5.15	4.10	4.58
24	6.15	5.05	4.40	5.20
Total	17.75	18.30	16.50	17.51
MILK				
6	0.05	0.02	0.08	0.05
12	0.12	0.11	0.15	0.13
18	0.16	0.12	0.13	0.14
24	0.13	0.17	0.14	0.15
Total	0.46	0.42	0.50	0.47

FIG. 31 . CUMULATIVE URINARY EXCRETION OF ^{3}H-AFB_1 RADIOACTIVITY AFTER SINGLE ORAL DOSE.



it excretable as a glucuronide or sulphate (Dalezios et al., 1972).

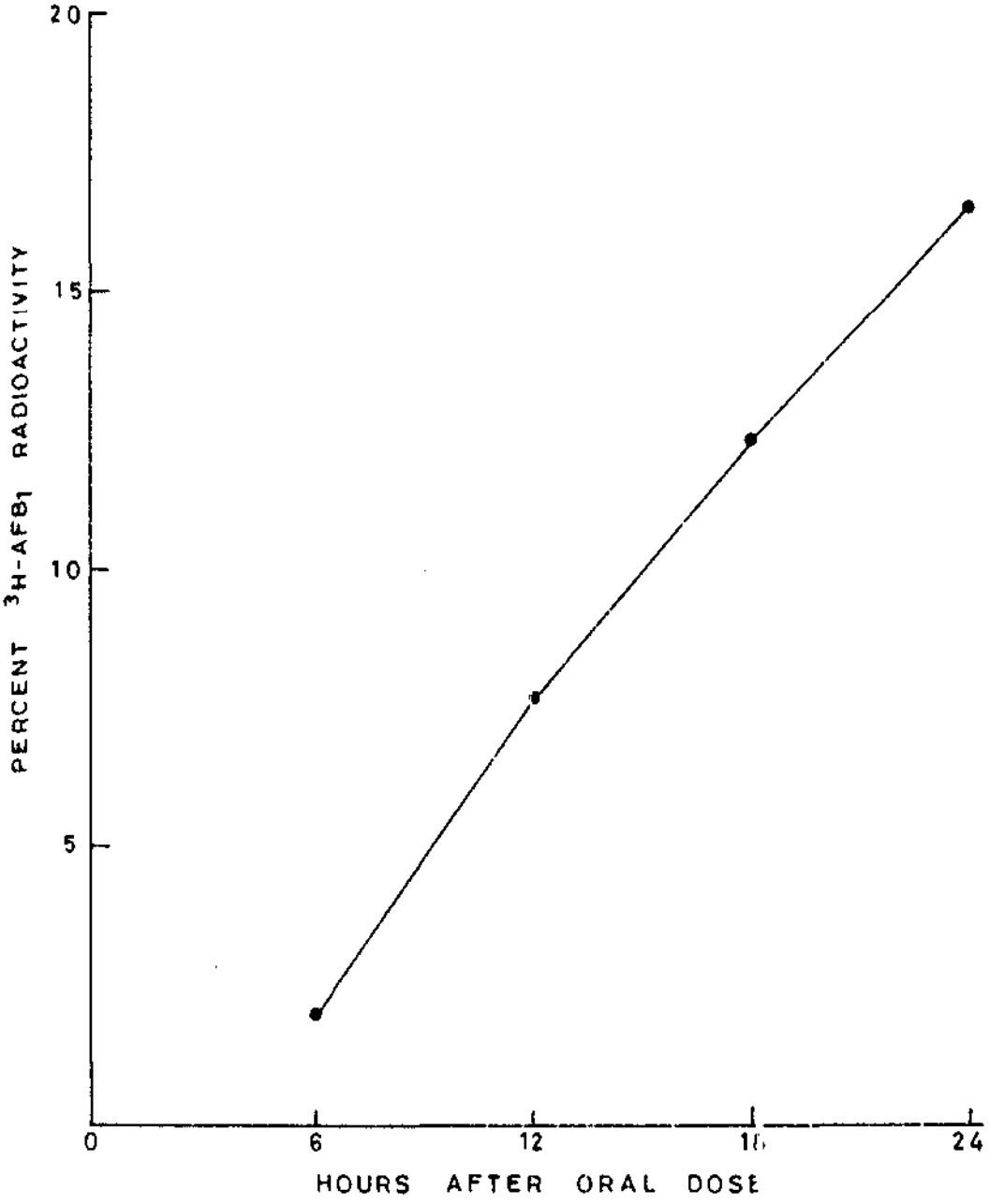
7.2.3.2 Faeces:

Recovery of radioactivity expressed as percent of administered dose from faeces in lactating goats at different time intervals is presented in Table 48 and depicted in Fig. 32. The average cumulative excretion of the radioactivity in the three lactating goats was 17.51 ± 0.53 per cent indicating a significant amount of radioactivity excreted through faeces in 24 h. Helferich et al. (1986) reported that the major route of elimination of aflatoxin is via faeces with 50 to 70 per cent being excreted in the goat and 48 to 78 per cent in gut plus faeces in the lactating rat in 120 h. They further reported that the transfer of radioactivity to faeces in both the species is presumably via biliary secretion and which is not reabsorbed (Wong, 1982). Wogan et al. (1967) observed that about 90 per cent of the single intra-peritoneal dose of AFB_1 was excreted or present in the intestinal tract contents within 24 h. But Polan et al. (1974) could detect only 5 per cent of the orally administered radioactivity (^3H-AFB_1) in faeces in 96 h in lactating cows. This indicated a wide variation in the excretion pattern of aflatoxin in different species, inspite of the fact that major route of excretion of aflatoxin is via faeces.

7.2.3.3 Milk:

Milk is the compartment of primary concern for human

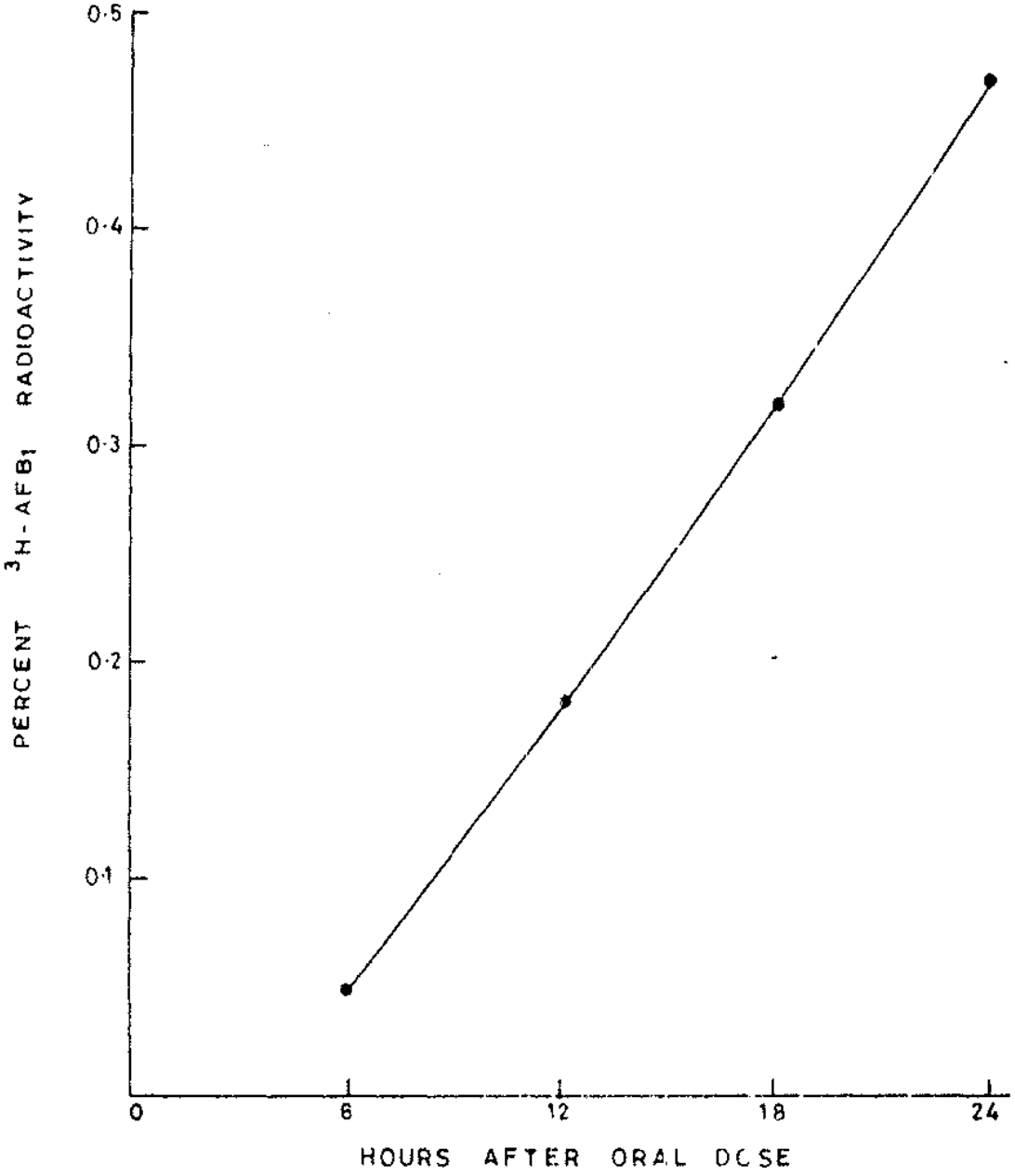
FIG. 32. CUMULATIVE FAECAL EXCRETION OF ^{3}H-AFB$_1$ RADIOACTIVITY AFTER SINGLE ORAL DOSE



risk assessment. The process of milk synthesis and secretion actually concentrates AFM_1 from blood against a concentration gradient (Helferich et al., 1986). The excretion of radioactivity expressed as percent of administered dose through milk in 3 lactating goats at different time intervals is presented in Table 48 and depicted in Fig. 33. The average cumulative excretion of radioactivity in milk in 24 h was 0.47 ± 0.02 per cent, a very small fraction of the administered radioactivity which indicated that milk is not the primary route of excretion of aflatoxins. Goto and Hsieh (1985) reported that milk collected in first 24 h following low dosing of ^{14}C-AFB_1 contained radioactivity equivalent to 0.45 to 1.1 per cent of the dose given in goats. Polan et al. (1974) could recover only 1.7 per cent of the radioactivity in milk, as cumulative percentage of administered dose and concluded on the basis of radioactivity and chemical analysis of milk that milk was not the principal excretory channel for aflatoxin. Cumulative recovery of AFB_1 metabolites in milk after 120 h was approximately 1 per cent (Helferich et al., 1986). They further reported that excretion rates of aflatoxin in lactating rats and goats were similar, although the lactating goats appeared to have greater conjugation capacity.

Excretion of AFM_1 by the cow occurs relatively quickly (Lancaster, 1969). Allcroft and Roberts (1968) reported that AFM_1 appeared in cow milk within 5 h of an orally administered dose of purified B_1. In this experiment also, radioactivity was detected in milk of all the three lactating

FIG. 33. CUMULATIVE EXCRETION OF ^{3}H-AFB$_1$ IN MILK AFTER SINGLE ORAL DOSE



goats at 6 h post-dosing. The reason for the rapid appearance of AFM_1 in milk may be the rapid transport of AFB_1 from the rumen. This is supported by the observation of Engel and Hagemeister (1978) who recovered only 15 per cent of AFB_1 administered into the rumen of cattle at the duodenum and only 2 to 5 per cent of the fed toxin reached the intestinum, suggesting significant absorption from the rumen. Similarly, Cook et al. (1986) observed aflatoxin M_1 in rumen contents from steers 2 h after an oral administration of aflatoxin and pointed out the intra-ruminal metabolism of aflatoxin B_1 to M_1.

7.2.4 Tissue distribution:

The weight of different organs and distribution of radioactivity as a percent of total administered dose in three goats are presented in Table 49. The average radioactivity in liver of three goats was 37.11 ± 0.91 per cent of the radioactive ^3H-AFB_1 administered orally, 24 h post-dosing, which was highest among all other organs observed for radioactivity in this experiment. Kidney was having 8.32 ± 0.56 per cent radioactivity of the administered dose. The other organs were having percent radioactivity of the administered dose as: spleen 1.69 ± 0.18; lungs 1.91 ± 0.12, heart 1.36 ± 0.20; ovary 0.57 ± 0.11; mammary gland 2.40 ± 0.35; pancreas 1.18 ± 0.19 and gall bladder 2.83 ± 0.17. The other organs were having radioactivity in the range of 0.57 to 2.83 per cent of the administered dose 24 h post-dosing.

Wogan et al.(1967) reported that radioactivity derived

Table 49. Weight of organ and percent radioactivity present of the administered dose

Organ	Goat Number						Average	
	381		657		677			
	Weight (g)	Radio-activity as percent of dose	Weight (g)	Radio-activity as percent of dose	Weight (g)	Radio-activity as percent of dose	Weight (g)	Percent radio activity
Liver	625.00	35.45	470.00	37.30	545.00	38.57	546.70 ± 44.75	37.11 ± 0.91
Kidney	130.50	7.50	100.00	9.40	91.50	8.06	107.33 ± 11.57	8.32 ± 0.56
Spleen	80.00	1.55	70.20	2.05	49.80	1.48	66.67 ± 8.82	1.69 ± 0.18
Lung	390.20	2.15	290.00	1.75	287.80	1.84	322.67 ± 33.67	1.91 ± 0.12
Heart	180.00	1.26	185.00	1.06	165.10	1.75	176.67 ± 6.01	1.36 ± 0.20
Ovary	2.65	0.70	1.66	0.65	2.08	0.35	2.40 ± 0.37	0.57 ± 0.11
Mammary gland	540.30	2.50	599.80	2.95	620.00	1.74	586.67 ± 24.03	2.40 ± 0.35
Pancreas	60.00	1.20	70.50	1.50	54.50	0.85	61.67 ± 4.41	1.18 ± 0.19
Gall bladder	25.25	3.15	24.75	2.75	20.00	2.58	23.33 ± 1.67	2.83 ± 0.17

from ^{14}C-AFB$_1$ in rats was present at maximum levels in liver and kidney 0.5 h after administration with small amounts present in other organs. The concentration of radioactivity was five to fifteen times greater in liver than in other tissues and at the end of 24 h, the liver contained an amount equal to the content of the remainder of the carcass.

Polan et al. (1974) reported that 85 per cent of the radioactivity administered orally as ^{3}H-AFB$_1$ in lactating cow was retained in various tissues and only 15 per cent was excreted in 96 hours.

Jarvis (1976) reported the presence of aflatoxin in liver, kidney and other tissues of pig fed diets having aflatoxin. Levels were highest in liver and kidney with only trace amount occurring in heart, muscle and adipose tissue.

Stubblefield et al. (1983) found AFB$_1$ and M$_1$ in all samples, except the thymus gland of a cow given aflatoxin B$_1$ orally at 0.35 mg/kg body weight for 3 days and sacrificed after 24 h of the last dose. Kidney, liver and mammary gland had the highest concentration of total aflatoxins.

Pelferich et al. (1986) could detect only traces of radioactivity from tissues of goats given an oral dose of ^{14}C-AFB$_1$ after 120 h of administration, with the exception of liver, indicating clearance from various tissues as a function of time.

High radioactivity observed in liver of this experiment after 24 h was an indication that liver was the primary

site of aflatoxin metabolism and 24 h period was not sufficient to metabolize the absorbed aflatoxin by the liver. Further studies are needed in different species and human beings to elucidate the time for complete elimination of aflatoxin from various tissues to avoid the potent danger of carcinogenesis.

CHAPTER 8

SUMMARY AND CONCLUSIONS

8. SUMMARY AND CONCLUSIONS

Aflatoxins are now known to cause toxicity of varying degrees in different species of livestock. These toxins are not only harmful to animals but equally dangerous to human beings because of consumption of animal originated foods such as milk, meat and egg. Studies on the role of aflatoxins in relation to growth and metabolism of farm animals; their interaction with various dietary constituents; interference with disease resistance; their tissue distribution and excretion pattern will be most valuable which may form the basis for determining the safe dietary levels of aflatoxins and may help to provide norms towards quality control measures. Keeping in view the above objectives, the present studies were undertaken on the following aspects:

1. To determine the protective effect of selenium on cellulose digestion and protein synthesis in aflatoxin containing medium.

2. To find out the aflatoxin toleration limit in buffalo calves and kids.

3. To study the effect of aflatoxin on nitrogen dynamics in cattle and buffalo.

4. To investigate the tissue distribution and excretion pattern of aflatoxins in lactating goats.

Experiment 1

8.1 EFFECTS OF AFLATOXIN ON IN VITRO RUMEN MICROBIAL ACTIVITY AS INFLUENCED BY DIFFERENT LEVELS OF SELENIUM

An *in vitro* experiment was conducted to study the protective effect of three levels of selenium (0, 0.25 and 0.5 ppm) against the adverse effect of three levels of aflatoxin (0, 0.25 and 0.5 ppm) on rumen microbial activity. A male Murrah buffalo calf aged 3 years and weighing 350 kg fitted with a permanent rumen fistula served as the donor of rumen liquor. McDougall's buffer was used in the medium. Cellulose and glucose served as carbohydrate source, and urea and ammonium sulphate were used as the sources of nitrogen for the growth of microbes. $Na_2{}^{35}SO_4$ carrier free, radioisotope was added in the medium to study the incorporation of sulphur into microbial protein. The fermentation was carried out for 24 h at 39 ± 1°C with 20 ml SRL in a 150 ml conical flask fitted with tight rubber cork having Bunsen gas release valve under anaerobic conditions.

The results with three respective selenium levels without aflatoxin were: cellulose disappearance (%): 31.64, 30.91, 30.42; TVFA production (mM/100 ml SRL): 2.18, 2.20, 2.20; microbial protein synthesis (mg): 20.99, 19.53, 20.99; ^{35}S-incorporation into microbial protein (%): 30.65, 29.90, 29.40. These results indicated that none of the selenium levels used in this study had any effect on rumen microbial activity. Cellulose disappearance rates at

0.25 ppm aflatoxin with 3 different levels of selenium were 21.17, 16.87 and 17.62 per cent while the values were 20.66, 12.32 and 15.48 per cent at 0.5 ppm aflatoxin in the artificial rumen, showing percent inhibition ranging from 33.09 to 46.68 at 0.25 ppm level and 34.70 to 61.06 at 0.5 ppm aflatoxin level.

Total volatile fatty acids concentration values at 0.25 ppm aflatoxin with 3 different levels of selenium were 1.08, 1.06 and 1.07 mM/100 ml SRL, while the values were 1.11, 0.81 and 0.33 at 0.5 ppm aflatoxin. These results indicated percent inhibition from 50.46 to 51.38 at 0.25 ppm level and 49.08 to 84.86 with 0.5 ppm level of aflatoxin.

Net microbial protein synthesis at 0.25 ppm aflatoxin was 9.84, 6.93 and 9.57 mg, while the values were 7.59, 3.59 and 3.60 mg at 0.5 ppm aflatoxin level. The corresponding inhibition with different levels of aflatoxin and selenium were 53.12, 66.98, 54.41, 63.84, 82.90 and 82.85 respectively. Incorporation of ^{35}S into microbial protein was 20.16, 15.86 and 16.62 at 0.25 ppm aflatoxin while the values were 19.65, 11.31 and 14.47 at 0.5 ppm aflatoxin. The corresponding inhibition in ^{35}S-incorporation with two levels of aflatoxin and levels of selenium was 34.23, 48.25, 45.77, 35.89, 63.10 and 52.79.

These results indicated that rumen microbial activity estimated in terms of cellulose disappearance, total volatile fatty acid concentration, net microbial protein synthesis and ^{35}S-incorporation into microbial

protein was significantly depressed ($P < 0.01$) as a result of aflatoxin toxicity on the rumen microbes. It was also evident that different levels of selenium used in this study did not protect the rumen microbes from the adverse effects of aflatoxin.

Experiment 2

8.2 EFFECTS OF AFLATOXIN ON GROWTH IN KIDS AND ITS INFLUENCE ON FEED CONVERSION EFFICIENCY

Twenty four male growing kids of Alpine, Saanen and Beetal crosses were divided into 4 groups of 6 kids each. One group was maintained as control group (1) and other three groups were fed aflatoxin at 0.3, 0.6 and 1.0 ppm (2 to 4) respectively on the basis of their dry matter consumption for a period of 16 weeks. Weekly weight gains and feed intake were recorded during this period.

The average daily weight gain (g) of kids in different treatment groups: Group 1: 72.63, Group 2: 58.36, Group 3: 47.15 and Group 4: 32.66 indicating decline in weight gain with increase in aflatoxin dosage. The effect of aflatoxin was not noticed in any group upto 11 weeks. From 12 weeks onwards, the kids of group 4 showed a depression in weight gain ($P < 0.05$); and from 13 weeks onwards in group 3. Cumulative weekly weight gains were accordingly less and varied with the dosage of aflatoxin toxicity. Dry matter consumption did not vary significantly

among various treatment groups. The results revealed impaired metabolism of nutrients at the absorption site or at the tissue level which resulted in retarded growth and reduced feed efficiency. Feed to gain ratios in different treatment groups were: Group 1: 8.95, Group 2: 10.48, Group 3: 12.60 and Group 4: 17.39, indicating higher dry matter consumption per unit live weight gain with increase in aflatoxin levels. From regression analysis of daily weight gains in different groups, it was evident that 0.14 ppm aflatoxin level might be safe for kids since at this level the daily weight gain of 67 g was not different from a gain of 72 g in control group.

Experiment 3

8.3 EFFECTS OF AFLATOXIN ON IMMUNOGLOBULIN STATUS OF BLOOD AND GROWTH IN BUFFALO CALVES

Twenty four male Murrah buffalo calves, weaned at birth were randomly distributed into 4 groups of six calves each at 10 days of age. Group 1 served as control while the other 3 groups (2-4) were fed aflatoxin at 0.3, 0.6 and 1.0 ppm respectively on the basis of their dry matter intake through milk (10 days of age to 15 weeks, Phase I) or concentrate mixture plus green fodder (18 weeks to 30 weeks, Phase II). Fortnightly blood samples were collected for the analysis of immunoglobulin in Phase I and weekly body weights were taken to assess their growth rate during both the phases.

8.3.1 Effect of aflatoxin on Ig

The initial values of Ig (mg/ml blood serum) on day 10 were 25.39, 28.59, 23.78 and 24.82 respectively in 4 groups which were not different statistically from each other, but there was a significant ($P < 0.01$) decline in Ig level with incremental increase in aflatoxin level. The decline in Ig level in blood serum of buffalo calves started from day 40, 55 and 100 of age given aflatoxin at 1.0, 0.6 and 0.3 ppm level respectively. The mean Ig concentration (mg/ml blood serum was 29.21, 21.67, 17.03 and 10.51 in 4 groups respectively. Probably aflatoxin affected the intrinsic synthesis of aflatoxin in these groups which led to continuous decline in Ig level. The effect was more pronounced at higher levels of aflatoxin than lower level.

8.3.2 Effect on growth rate and feed conversion efficiency (Phase I)

The average daily weight gain (g) in different treatment groups were: Group 1: 402.3, Group 2: 318.5, Group 3: 264.3 and Group 4: 205.7, indicating the decline in weight gain with increase in aflatoxin levels. Statistical analysis of the data indicated that weekly weight gains were significantly reduced ($P < 0.01$) from 3rd week onwards in all the aflatoxin fed groups. Cumulative weekly weight gains were accordingly less and varied with the level of aflatoxin toxicity. Dry matter consumption did not differ among various treatment groups. Feed to gain ratios were: Group 1: 1.34, Group 2: 1.65, Group 3: 2.14

and Group 4: 2.69, indicating higher dry matter consumption per unit weight gain with increase in aflatoxin level. Through regression analysis of daily weight gains, it was inferred from growth curve that 0.13 ppm aflatoxin might be safe for pre-ruminant buffalo calves.

8.3.3 Effect of aflatoxin on growth and feed conversion efficiency (Phase II)

The effect of feeding aflatoxin on buffalo calves (post-ruminant stage) was studied for 12 weeks, keeping the same grouping as in Phase I. The average daily weight gains (g) in different treatment groups were: Group 1: 400, Group 2: 295, Group 3: 225 and Group 4: 202, indicating a decline in weight gain with the increase in aflatoxin level. Analysis of variance of the data revealed that weekly weight gains were reduced from 4th week onwards in groups 3 and 4 and from 9th week onwards in group 2. There was no effect of feeding aflatoxin on dry matter intake. Feed:gain ratios were 6.15, 8.62, 11.41 and 12.10 in 4 groups respectively, indicating higher dry matter consumption per unit weight gain with increase in aflatoxin level. The safe level of aflatoxin estimated by drawing a regression line on the basis of growth was found to be 0.15 ppm.

Since there was no difference in the DM intake of different groups in both phases, it was thus derived that impaired metabolism of nutrients at the absorption site or utilization at the tissue level was responsible for retarded growth and reduced feed efficiency in buffalo calves fed graded levels of aflatoxin.

Experiment 4

8.4 EFFECT OF AFLATOXIN ON NITROGEN DYNAMICS IN CATTLE AND BUFFALO CALVES FED DIFFERENT LEVELS OF DIETARY PROTEIN

An experiment was conducted on 8 male rumen fistulated, i.e., 4 cattle and 4 buffalo calves and completed in two phases. Four crossbred calves were assigned to groups 1, 2, 5 and 6 and similarly 4 buffalo calves were allotted to groups 3, 4, 7 and 8, one animal representing each group. All the animals were fed on concentrate mixture, green fodder and wheat straw to meet their nutrient requirements. Animals in groups 1, 2, 3 and 4 were given 42.5 per cent more CP (HP) in phase I and optimum protein (OP) in phase II, while the animals in groups 5, 6, 7 and 8 were provided optimum CP (OP) in phase I and 51.47 per cent less protein (LP) than optimum in phase II. In addition, animals in groups 2, 4, 6 and 8 were given an oral dose of aflatoxin @ 1.0 ppm of their dry matter intake.

Phase I

8.4.1 Studies on digestibility of nutrients

After adapting the animals for a period of 40 days, a metabolism trial was conducted to estimate the digestibility of nutrients. The digestibility coefficient of DM, OM and crude protein were in Group 1: 69.08, 72.62, 71.37; Group 2: 64.45, 68.03, 73.85; Group 3: 69.70,

72.46, 73.86; Group 4: 67.60, 71.21, 73.48; Group 5: 63.27, 66.03, 64.43; Group 6: 66.29, 69.44, 64.93; Group 7: 69.22, 72.75, 69.06 and Group 8: 68.18, 72.16, 68.13. The results indicated that aflatoxin had no effect on the digestibility of these nutrients. However, the digestibility of crude protein was more in both the species fed higher level of protein. There was no species difference in the digestibility of crude protein. The digestibility of other nutrients was alike in all the groups

8.4.2 Nitrogen balance studies

The average N intakes were 179.35 and 125.86 g/day respectively in HP and OP fed groups which indicated an average of 42.5 per cent more N intake in groups 1-4. The data revealed that out of 179.8, 179.8, 179.8, 178.0; 126.5, 125.46, 126.0, 125.5 g N intake per day respectively in groups 1 through 8; 51.47, 47.0, 47.0, 47.2, 45.0, 44.0, 39.0 and 40.0 g of nitrogen was excreted daily in the faeces. Likewise, the nitrogen excretion pattern through urine was 55.20, 59.40, 32.17,59.24, 34.30, 38.66, 23.09 and 32.83 as per cent of nitrogen intake in groups 1 through 8. Correspondingly, nitrogen retention was 29.07, 25.99, 39.0, 25.40, 38.10, 32.96, 57.9 and 44.30 g N per day amounting to 16.17, 14.45, 21.69, 14.27, 30.12, 26.27, 45.95 and 35.30 per cent of nitrogen intake. Results indicated that all the animals were in positive nitrogen balance but N balances (g/d) were more in buffalo calves as compared to crossbred calves. The quantity of N retained was less in aflatoxin fed calves of both the species irrespective of level of protein.

8.4.3 Infusion studies with ^{15}N-ammonium sulphate and ^{51}Cr-EDTA

The daily dietary feed requirements were divided into six equal parts and were fed at two hourly intervals during first 12 hours of the day for 10 days, after which ^{51}Cr-EDTA and ^{15}N-ammonium sulphate were administered into the rumen of each animal as a single injection. Samples of rumen liquor, urine and blood were collected at different time intervals to study the effect of feeding aflatoxin on water and ammonia flow from the rumen and nitrogen dynamics in these animals fed HP or OP diets.

8.4.4 Effect of aflatoxin on water and ammonia flow from the rumen

Using ^{51}Cr-EDTA, the rumen volumes in the animals were 37.70, 56.00, 45.38, 55.49, 45.71, 54.90, 50.46 and 43.77; rumen fluid outflow rates were 87.34, 90.20, 105.90, 123.70, 93.95, 88.14, 89.88 and 107.78 l/day in groups 1 through 8 respectively. Corresponding values for ammonia outflow from rumen fluid were 19.39, 21.87, 27.85, 29.07, 10.09, 12.08, 9.35 and 12.18 g N/day, indicating higher outflow of ammonia-N in HP fed groups (1 to 4), related to higher nitrogen intake.

8.4.5 Effect of aflatoxin on kinetics of ammonia in rumen and blood of calves and buffalo calves fed HP and OP diets

The values of ruminal-NH_3 pool size in different groups were: Group 1: 4.63, Group 2: 3.96, Group 3: 4.87,

Group 4: 4.32, Group 5: 2.90, Group 6: 2.30, Group 7: 2.83 and Group 8: 3.06 g N. The ruminal-NH_3 entry rates were 27.35, 30.36, 37.12, 36.47, 18.26, 21.23, 20.21 and 19.76 g N/day ; ruminal-NH_3 irreversible loss rates were: 19.01, 21.35, 25.77, 26.91, 12.59, 14.09, 12.74 and 13.25 g N/day and recycling rates were 8.34, 9.01, 11.35, 9.56, 5.67, 7.14, 5.47 and 5.51 g N/day in groups 1 through 8, respectively.

The values for ruminal-NH_3 contribution to suspended bacterial-N, plasma urea-N and urinary-N in different groups were: Group 1: 42.85, 36.38, 34.15; Group 2: 38.41, 39.17, 38.20; Group 3: 38.62, 39.45, 20.42; Group 4: 33.75, 38.40, 25.44; Group 5: 49.81, 32.20, 20.57; Group 6: 50.62, 30.25, 23.85; Group 7: 47.24, 29.40, 16.41 and Group 8: 49.42, 35.85 and 19.80.

Higher percent incorporation of ruminal-NH_3 into bacterial-N in OP fed groups, as compared to HP fed groups irrespective of aflatoxin intake, indicated a better utilization of N by rumen microbes, when less amount of N was available. Similarly, plasma urea-N derived from ruminal-NH_3 was higher in animals fed HP diets as compared to OP fed groups in both the species, without any aflatoxin effect. Results also indicated that buffalo calves were superior to crossbred calves as the former had more positive nitrogen balances; and percent N utilization of the absorbed was better in tissues with both regimens.

Phase II

8.4.6 Effect of aflatoxin on digestibility of nutrients

Digestibility of all the nutrients was less in LP fed animals, irrespective of species and aflatoxin intake.

8.4.7 Effect of aflatoxin on nitrogen balance

The average N intake (g/day) was 128.67 and 62.49 respectively in OP and LP groups, which indicated that 51.47 per cent less nitrogen was provided to animals in the latter groups as compared to OP groups. The data revealed that out of 130.11, 123.02, 132.80, 129.10, 61.72, 62.93, 62.30 and 63.04 g N intake per day respectively, 45.63, 43.75, 50.0, 44.38, 37.50, 36.89, 34.37 and 33.0 g N/day was excreted daily in the faeces of groups 1 through 8. Likewise, the nitrogen excretion pattern through urine was 45.83, 52.84, 33.51, 39.55, 37.27, 40.76, 34.51 and 43.22 per cent of nitrogen intake in groups 1 through 8. Correspondingly, nitrogen retention was 14.85, 14.27, 38.30, 33.66, 1.22, 0.48, 6.43, 2.79 g N/day, the values amounted to 19.10, 11.60, 28.84, 26.07, 1.98, 0.76, 10.32 and 4.43 per cent of the N intake.

Inspite of providing 51.47 per cent less protein to LP groups, the animals showed positive N balances, though the values were much higher in OP groups in both the species. It also appeared from the data that aflatoxin led to decrease in nitrogen balances in both the species of animals irrespective of dietary protein intake.

8.4.8 Effect of aflatoxin on water and ammonia outflow from the rumen

Rumen volumes as estimated by using ^{51}Cr-EDTA as a marker were: 39.50, 57.45, 48.50, 56.40, 45.50, 55.20, 51.0 and 43.50. Rumen fluid outflow rates were 85.40, 95.20, 101.40, 120.60, 92.50, 90.00, 91.50 and 110.25 l/day in groups 1 through 8 respectively. The values for ammonia outflow from rumen fluid were 11.72, 12.06, 14.20, 17.13, 5.70, 6.48, 5.23 and 6.86 g N/day in groups 1 through 8 respectively, indicating again that higher N outflow in groups 1 to 4 was related to higher N intake.

8.4.9 Effect of aflatoxin on the kinetics of ammonia in rumen and blood

The ruminal-NH_3 pool sizes in different groups were: Group 1: 3.30, Group 2: 2.85, Group 3: 3.12, Group 4: 2.90, Group 5: 1.94, Group 6: 1.81, Group 7: 2.16 and Group 8: 2.23 g N/day. The values for ruminal-NH_3 entry rates were 25.87, 24.07, 21.05, 19.65, 15.26, 13.45, 15.44 and 16.57 g N/day; ruminal-NH_3 irreversible loss rates were: 14.48, 18.06, 12.95, 12.86, 9.87, 9.35, 9.56 and 11.05 g N/day and recycling rates were 11.39, 6.01, 8.10, 6.79, 5.39, 4.10, 5.88 and 5.52 g N/day in groups 1 to 8 respectively. Higher values for ruminal-NH_3 pool and ruminal-NH_3 entry rates in groups 1 to 4 were due to more protein intake and aflatoxin did not affect these parameters.

The values for ruminal-NH_3 percent contribution to suspended bacterial-N, plasma urea-N and urinary-N

in different groups were: Group 1: 52.64, 36.38, 24.35; Group 2: 51.50, 39.46, 28.20; Group 3: 53.60, 32.56, 18.20; Group 4: 49.54, 33.48, 24.45; Group 5: 61.75, 25.15, 20.75; Group 6: 65.87, 22.28, 25.78; Group 7: 70.76, 18.20, 19.47 and Group 8: 71.87, 19.26, 28.32.

Results obtained in both the phases indicated that there was no effect of feeding aflatoxin on digestibility of nutrients. Digestibility of nutrients was more in groups fed OP diets as compared to LP groups. Nitrogen balances (g/day) were positive in all the animals irrespective of level of protein and species. Nitrogen excretion through urine (g/day) was more in calves and buffalo calves fed aflatoxin. Buffalo calves seemed to be superior over calves showing more positive N balances and better utilization of absorbed N.

Results further showed that contribution of ruminal-NH_3 towards bacterial protein and plasma urea-N synthesis was inversely and directly related with the nitrogen intake, respectively indicating better utilization of ruminal-NH_3 when less N was available. The contribution of ruminal-NH_3 towards urinary-N was more in animals fed HP than OP; and optimum protein fed animals had more contribution of ruminal NH_3 towards their urinary-N than LP fed groups. Aflatoxin led to more excretion of ruminal ammonia-N into urinary-N and that too more in cattle than buffalo calves fed any level of protein.

Experiment 5

8.5 TISSUE DISTRIBUTION AND EXCRETION PATTERN OF AFLATOXIN IN LACTATING GOATS

Experiment was conducted on 12 lactating goats divided into 2 groups. All the goats were fed on concentrate mixture and green maize. In addition, goats in group 2 (experimental) were given aflatoxin @ 1.0 ppm of their dry matter intake. After adapting the animals on this dietary regimen, a metabolism trial was conducted to know the digestibility of nutrients. After the metabolism trial was over, 3 goats from the experimental group were given ^{3}H-AFB_1 (200 µCi/goat) and excretion of radioactivity was studied at different hours in faeces, urine and milk upto 24 hours post-dosing. After 24 hours, these goats were sacrificed and various tissues were removed to study the distribution of ^{3}H-AFB_1.

8.5.1 Digestibility of nutrients

Results indicated that there was no effect of feeding aflatoxin at 1.0 ppm level on any of the digestibility of proximate principles but dry matter intake kg $W^{0.75}$ was significantly ($P < 0.01$) reduced due to aflatoxin feeding in group 2.

8.5.2 Nitrogen balance

The average N intake in groups 1 and 2 was 26.28 and 18.32 g/day. The data revealed that there was no effect of aflatoxin on faecal N excretion whe calculated

as a percent of nitrogen intake. The excretion of N through urine was 7.83 and 8.56 g/day in groups 1 and 2 respectively which was 30.13 and 43.46 per cent of the nitrogen intake and indicated a significant ($P < 0.01$) effect of aflatoxin on N excretion through urine. Excretion of N through milk was unaffected due to aflatoxin intake. Goats in both the groups were in positive N balance but the quantity of N balance was significantly ($P < 0.01$) higher in control group as compared to aflatoxin group. N retained as percent of absorbed N as such and calculated on per kg live weight basis was significantly ($P < 0.01$) more in group 1 than group 2, indicating poor utilization of N due to aflatoxin intake.

8.5.3 Excretion pattern of ^{3}H-AFB_1

8.5.3.1 Urine:

The average excretion of radioactivity through urine in 3 goats was 0.37, 1.83, 2.71, 3.39 and 4.57 per cent of the oral dose at 3, 6, 12, 18 and 24 hour post-dosing respectively. The cumulative excretion of radioactivity in urine in 24 hours was 12.93 per cent of the administered dose. Detection of radioactivity at 3 hours post-aflatoxin dosing indicated a rapid absorption of the aflatoxin from the gastro-intestinal tract.

8.5.3.2 Faeces:

The average excretion of radioactivity through faeces in 3 goats was 2.03, 5.70, 4.53 and 5.20 per cent of the oral dose at 6, 12, 18 and 24 hour respectively.

The cumulative excretion in 24 hours was 17.51 per cent of the administered dose of ^{3}H-AFB_1. The appearance of radioactivity in faeces at 6 hour post dosing indicated its faster passage in the gastro-intestinal tract. Faeces appeared to be the primary channel of aflatoxin excretion.

8.5.3.3 Milk:

The average secretion of radioactivity in milk in 3 goats was 0.05, 0.13, 0.14 and 0.15 per cent of the dose at 6, 12, 18 and 24 hour respectively. The cumulative secretion of radioactivity in the milk was 0.47 per cent of the administered dose, a very small fraction, which indicated that milk is not the primary route of aflatoxin excretion.

8.5.4 Tissue distribution

The average radioactivity in various tissues was: liver: 37.11, kidney: 8.32, spleen: 1.69, lungs: 1.91, heart: 1.36, ovary: 0.57, mammary gland: 2.40, pancreas: 1.18 and gall bladder: 2.83 per cent of the administered dose. Liver was having the highest radioactivity followed by kidney and other organs were having the radioactivity in the range of 0.57 to 2.83 per cent of the administered dose. Liver seemed to be the primary site of aflatoxin action where it was retained significantly even upto 24 hour period.

CHAPTER 9

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BIBLIOGRAPHY

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CHAPTER 10

A P P E N D I C E S

Table A1. Effect of different levels of aflatoxin and selenium on *in vitro* cellulose disappearance

Treatment No.	Treatment		Replicate			
	Se (ppm)	Af (ppm)	1	2	3	Average ± S.E.
1	0.0	0.0	27.86	33.55	33.50	31.64 ± 1.89
2	0.25	0.0	26.77	32.45	33.50	30.91 ± 2.09
3	0.50	0.0	27.17	31.45	32.66	30.42 + 1.67
4	0.0	0.25	25.65	17.32	20.55	21.17 ± 2.42
5	0.25	0.25	22.04	15.60	12.96	16.87 ± 2.70
6	0.50	0.25	10.48	19.02	23.37	17.62 ± 3.78
7	0.0	0.50	14.85	19.97	27.17	20.66 ± 3.57
8	0.25	0.50	12.96	9.15	14.84	12.32 ± 1.67
9	0.50	0.50	9.16	15.99	21.30	15.48 ± 3.51

Table A2. Effect of different levels of aflatoxin and selenium on in vitro TVFA concentration

Treatment No.	Treatment		Replicate			
	Se (ppm)	Af (ppm)	1	2	3	Average ± S.E.
1	0.0	0.0	2.25	1.95	2.35	2.18 ± 0.12
2	0.25	0.0	2.30	2.20	2.10	2.20 ± 0.06
3	0.50	0.0	2.20	2.45	1.95	2.20 ± 0.14
4	0.0	0.25	1.00	0.73	1.52	1.08 ± 0.23
5	0.25	0.25	0.78	0.87	1.53	1.06 ± 0.24
6	0.50	0.25	1.20	0.38	1.63	1.07 ± 0.37
7	0.0	0.50	0.93	1.30	0.28	1.11 ± 0.15
8	0.25	0.50	1.45	0.35	0.63	0.81 ± 0.33
9	0.50	0.50	0.38	0.03	0.60	0.33 ± 0.17

Table A3. Effect of different levels of aflatoxin and selenium on *in vitro* protein synthesis

Treatment No.	Treatment		Replicate			
	Se (ppm)	Af (ppm)	1	2	3	Average ± S.E.
1	0.0	0.0	20.57	24.32	18.07	20.99 ± 1.81
2	0.25	0.0	20.73	18.09	19.77	19.53 ± 0.78
3	0.50	0.0	21.51	22.60	18.85	20.99 ± 1.11
4	0.0	0.25	14.16	7.76	7.60	9.84 ± 2.16
5	0.25	0.25	12.29	6.82	4.32	6.93 ± 2.71
6	0.50	0.25	4.43	12.13	11.98	9.51 ± 2.54
7	0.0	0.50	5.26	6.95	10.57	7.59 ± 1.37
8	0.25	0.50	2.29	4.95	3.54	3.59 ± 0.77
9	0.50	0.50	1.51	4.90	4.37	3.59 ± 1.08

Table A4. Effect of different levels of aflatoxin and selenium on *in vitro* ^{35}S-incorporation

Treatment No.	Treatment		Replicate			
	Se (ppm)	Af (ppm)	1	2	3	Average ± S.E.
1	0.0	0.0	26.86	32.55	32.55	30.65 ± 1.90
2	0.25	0.0	25.76	31.44	32.50	29.96 ± 2.09
3	0.50	0.0	26.16	30.40	31.66	29.40 ± 1.66
4	0.0	0.25	24.65	16.31	19.53	20.16 ± 2.43
5	0.25	0.25	21.04	14.60	11.95	15.86 ± 2.70
6	0.50	0.25	9.48	18.02	22.36	16.62 ± 3.78
7	0.0	0.50	13.84	18.96	26.16	19.65 ± 3.57
8	0.25	0.50	11.95	8.15	13.84	11.31 ± 1.67
9	0.50	0.50	8.15	14.98	20.29	14.47 ± 3.51

Table A5. Weekly body weights of kids fed different levels of aflatoxin

Treatment group	Kid No.	Initial body wt (kg)	WEEKS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Gain in wt (kg)
Group I (0 ppm Af) Control	AB 674	10.2	10.5	11.0	11.5	12.0	12.3	13.0	13.6	14.4	14.0	14.6	14.8	15.2	15.8	16.0	16.5	17.0	6.8
	AB 678	11.0	11.2	11.4	11.5	11.8	11.8	12.4	12.7	14.0	13.5	14.3	14.7	15.1	16.0	16.4	17.0	17.4	6.4
	AB 681	11.2	11.5	11.8	11.8	12.1	12.4	13.6	14.0	14.4	14.8	15.2	16.4	16.0	17.2	18.0	19.0	19.2	8.0
	AB 689	11.8	12.3	13.0	13.4	13.9	13.9	14.2	15.0	14.4	15.4	15.8	16.4	16.6	17.6	17.6	18.8	19.0	7.2
	ASB 419	11.0	12.0	12.5	13.4	14.3	14.8	15.5	16.2	17.0	17.3	18.3	18.4	18.6	19.2	19.8	20.2	20.4	9.4
	SB 297	12.2	13.0	13.8	14.7	15.3	16.2	17.5	18.0	18.2	19.0	18.8	20.0	20.2	21.0	22.6	23.0	23.2	11.0
Group II (0.3 ppm Af)	AB 676	10.6	11.2	11.8	11.5	11.7	12.6	12.4	13.4	14.4	14.2	14.8	15.0	15.0	15.8	16.2	17.0	17.4	6.8
	AB 683	11.0	11.6	12.5	13.2	13.9	14.3	15.8	16.0	16.2	17.4	18.4	18.0	18.0	20.0	20.0	20.4	20.6	9.6
	AB 692	11.0	11.4	11.8	12.8	12.8	12.8	13.0	13.6	14.0	14.0	14.2	14.2	14.6	14.8	14.8	15.0	15.2	4.2
	SB 301	11.6	11.4	11.8	12.0	12.3	12.0	12.8	13.6	14.0	14.2	14.7	15.3	15.6	16.0	17.0	18.0	18.4	7.4
	SB 420	11.6	11.8	12.0	12.4	12.8	13.0	13.4	13.8	14.1	14.4	14.7	15.0	15.4	15.8	16.2	16.8	17.2	5.6
	SB 425	11.2	11.6	12.0	12.2	12.6	13.0	13.4	13.8	14.0	14.4	14.8	15.1	15.4	15.8	16.0	16.4	16.8	5.6

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Treatment group	Kid No.	Initial body wt (kg)	WEEKS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Gain in wt (kg)
Group 3 (0.6 ppm Af)	AB 680	11.0	11.3	11.8	11.5	11.8	12.5	13.4	13.8	14.1	14.0	14.4	15.4	14.6	15.6	15.8	16.0	16.8	5.8
	AB 688	12.4	12.8	13.2	13.6	14.0	14.4	14.6	15.0	15.4	15.8	16.0	16.2	16.4	16.8	17.0	17.4	17.8	5.4
	AB 691	10.0	10.2	10.7	10.5	10.8	11.0	12.2	12.2	12.4	12.8	13.2	13.4	13.2	13.8	14.0	14.6	14.8	4.8
	SB 298	10.8	11.0	11.5	11.3	11.0	11.2	11.5	11.5	11.3	11.4	11.8	12.0	11.6	12.2	12.2	12.8	13.8	3.0
	ASB 418	11.0	11.5	12.2	13.0	13.5	15.0	15.0	15.0	16.0	15.8	16.2	16.4	16.6	17.2	18.6	19.3	19.9	8.9
	ASB 424	11.0	11.4	12.0	12.6	13.0	13.5	14.0	14.0	14.2	14.2	14.8	15.0	14.4	14.4	15.0	14.6	14.8	3.8
	AB 677	10.8	11.2	11.6	12.0	12.4	12.8	13.0	13.4	13.6	13.8	14.0	14.4	14.8	15.0	15.2	15.6	15.8	5.0
roup	AB 685	11.2	11.5	12.0	12.2	12.0	11.5	12.3	11.4	12.0	12.3	12.6	12.8	12.6	12.8	12.4	12.8	12.8	1.6
1.0	AB 693	9.2	9.6	9.5	9.8	10.2	9.8	10.4	10.0	10.4	10.2	10.4	10.6	10.2	10.3	10.6	10.6	10.8	1.6
ppm Af)	SB 296	10.8	11.4	12.3	13.2	14.0	14.2	15.0	15.2	16.2	15.6	16.0	16.2	16.4	16.2	17.0	17.2	17.4	6.6
	ASB 423	11.2	11.6	12.0	11.8	12.2	12.7	13.8	13.0	14.0	14.2	14.4	14.8	15.0	15.2	16.2	16.8	16.2	5.0
	ASB 426	13.2	13.5	13.9	13.7	14.0	13.5	14.3	14.0	14.2	14.0	13.8	14.8	14.4	14.6	15.0	14.8	15.4	2.2

Table A6. Effect of feeding aflatoxin on immunoglobulin level in blood serum (mg/ml) of buffalo calves

Treatment group	Buffalo calf No.	Age (days)						
		10	25	40	55	70	85	100
Group 1 (0 ppm Af) Control	3157	13.12	27.89	36.92	52.48	59.52	60.92	35.61
	3158	49.68	41.25	38.43	27.19	27.19	29.98	29.98
	3161	24.88	42.09	44.00	35.61	27.16	25.77	27.16
	3166	6.10	10.32	20.72	14.52	14.52	18.74	21.55
	3186	17.33	25.77	32.80	27.17	18.73	21.55	21.55
	3206	41.23	35.61	21.55	21.55	21.55	18.76	15.93
Group 2 (0.3 ppm Af)	3153	44.06	41.25	32.81	27.17	24.36	17.37	20.14
	3159	18.75	27.19	23.67	18.73	31.39	27.17	28.42
	3169	15.93	31.40	25.06	25.77	20.99	35.61	15.93
	3176	7.50	12.60	13.11	13.11	25.77	6.08	8.90
	3179	46.86	38.42	27.17	13.11	15.93	18.73	13.11
	3184	38.42	7.49	27.17	20.14	18.76	15.93	10.26

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Treatment group	Buffalo calf No.	Age (days)						
		10	25	40	55	70	85	100
Group 3 (0.6 ppm Af)	3154	49.68	44.06	35.62	22.97	18.74	17.33	17.00
	3165	7.50	7.50	11.72	9.60	6.08	15.93	13.11
	3172	24.36	24.36	13.13	10.30	10.30	13.11	7.49
	3178	15.93	15.93	27.17	13.11	15.93	11.71	10.30
	3189	32.80	27.75	31.39	24.36	18.74	17.33	14.52
	3213	12.43	13.11	15.84	18.74	10.26	9.35	7.49
Group 4 (1.0 ppm Af)	3237	24.36	18.74	10.30	13.11	7.49	8.39	7.50
	3238	21.54	10.30	7.49	9.30	7.50	7.49	8.88
	3244	27.17	18.74	10.30	9.93	7.48	10.30	10.30
	3245	24.36	20.14	10.26	9.26	10.20	7.49	4.59
	3246	18.74	15.93	18.74	10.12	10.32	13.11	7.50
	3247	32.79	18.74	10.26	10.10	18.74	7.50	10.25

Table A7. Effect of feeding aflatoxin on weight gain in buffalo calves (Phase I)

Treatment group	Buffalo calf No.	Initial body wt (kg)	WEEKS													Weight gain (kg)
			1	2	3	4	5	6	7	8	9	10	11	12	13	
Group 1 (0 ppm Af) Control	3157	38.0	39.0	42.5	45.0	48.0	51.0	54.0	57.0	60.0	63.0	66.0	69.0	72.5	75.5	37.5
	3158	40.0	42.0	45.0	48.0	51.0	54.0	57.0	60.0	63.0	67.0	70.0	73.5	76.5	81.0	41.0
	3161	38.0	41.5	42.0	44.5	46.5	49.0	51.5	55.0	58.0	60.5	62.5	65.5	67.5	69.0	31.0
	3166	25.0	27.0	29.0	31.0	33.0	35.0	38.0	41.0	43.0	45.0	47.0	49.0	52.0	55.0	30.0
	3186	36.0	39.5	42.0	45.0	48.0	51.0	54.0	57.0	60.0	63.0	67.0	70.0	73.0	76.0	40.0
	3206	38.0	39.5	42.5	46.0	49.0	53.0	55.0	58.5	62.0	65.0	68.5	71.0	75.0	78.0	40.0
Group 2 (0.3 ppm Af)	3153	36.0	38.5	40.0	42.0	44.0	46.5	49.0	50.0	51.5	54.0	57.0	60.0	63.0	66.0	30.0
	3159	34.0	37.0	38.5	39.5	40.5	42.5	45.0	46.5	49.0	50.0	53.0	55.0	58.0	61.0	27.0
	3169	32.0	34.5	36.5	39.0	41.0	44.0	45.5	48.0	51.0	52.0	53.0	55.0	58.0	61.0	29.0
	3176	39.0	41.0	42.0	44.0	46.5	48.5	50.5	52.0	54.0	56.0	58.0	61.0	63.0	65.0	26.0
	3179	37.0	39.0	41.0	43.0	46.0	49.0	52.0	55.0	57.0	59.0	61.0	64.0	66.0	68.0	31.0
	3184	40.0	43.5	46.0	48.0	50.0	52.0	54.0	56.0	58.5	61.0	64.0	67.0	70.0	73.0	33.0

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Treatment group	Buffalo calf No.	Initial body wt (kg)	WEEKS 1	2	3	4	5	6	7	8	9	10	11	12	13	Weight gain (kg)
	3154	33.0	35.0	37.0	39.0	41.5	44.0	46.0	48.0	50.0	51.0	52.0	53.0	54.5	57.0	24.0
Group 3 (0.6 ppm Af)	3165	37.0	40.0	42.0	43.0	45.0	47.0	48.5	51.0	52.5	55.0	58.0	61.0	64.0	67.0	30.0
	3172	27.0	29.0	31.0	33.0	35.0	37.0	39.0	40.0	41.0	42.0	43.0	44.0	45.5	47.0	20.0
	3178	40.0	42.5	44.0	46.0	49.0	51.0	53.0	55.0	58.0	61.0	63.0	65.0	68.0	70.0	30.0
	3189	33.0	35.0	37.0	39.0	41.0	44.0	47.0	49.0	51.0	52.0	53.0	53.5	54.0	54.0	21.0
	3237	37.0	39.0	41.0	43.0	45.0	47.0	48.0	49.0	50.5	51.0	53.0	54.0	55.0	56.0	19.0
	3213	25.0	27.0	29.0	31.0	32.0	33.0	34.0	35.0	36.0	37.0	38.0	39.0	40.0	42.0	17.0
Group 4 (1.0 ppm Af)	3238	40.0	41.0	42.0	44.0	46.0	48.0	50.0	51.0	51.0	52.0	52.0	53.0	54.0	55.0	15.0
	3244	39.0	41.0	42.0	44.0	46.0	48.0	50.0	51.0	52.0	53.0	54.5	56.0	57.0	58.0	19.0
	3245	38.0	40.0	42.0	44.0	46.0	48.0	49.0	50.0	51.0	52.0	53.0	54.0	55.0	55.0	17.0
	3246	39.0	42.0	43.5	45.0	46.0	48.0	50.0	52.0	53.0	54.0	55.0	56.5	57.5	59.0	20.0
	3247	37.5	40.0	42.0	43.5	45.0	47.5	48.5	50.0	51.5	53.5	55.0	57.5	59.0	61.0	23.5

Table A8. Effect of feeding aflatoxin on weight gain in buffalo calves (Phase II)

Treatment group	Buffalo calf No.	Initial body weight (kg)	WEEKS												Weight gain (kg)
			1	2	3	4	5	6	7	8	9	10	11	12	
Group 1 (0 ppm Af) Control	3157	76.0	78.0	81.0	83.0	86.0	88.0	90.0	92.0	95.0	97.0	100.0	103.0	106.0	30.0
	3158	85.0	88.0	91.0	94.0	97.0	100.0	103.5	106.0	109.0	112.0	115.0	117.0	120.0	35.0
	3161	73.0	75.0	78.0	81.0	84.0	86.0	89.0	92.5	95.0	98.0	101.0	104.0	107.0	34.0
	3166	56.0	58.0	61.0	63.0	65.0	68.0	70.0	72.0	75.0	78.0	81.0	84.0	87.0	31.0
	3186	80.0	83.0	86.0	89.0	92.5	96.0	99.0	102.0	104.0	107.0	110.0	113.5	117.0	37.0
	3206	81.0	84.0	86.0	89.0	92.0	95.0	98.0	101.0	104.0	107.0	110.0	113.0	116.0	35.0
Group 2 (0.3 ppm Af)	3153	72.5	74.0	76.0	78.0	80.0	82.0	83.0	85.0	87.0	89.0	91.0	93.0	95.0	22.5
	3159	67.0	69.0	73.0	74.0	76.5	79.0	82.0	83.5	84.5	85.5	88.0	90.0	92.0	25.0
	3169	63.0	65.0	68.0	70.0	71.0	73.0	74.5	76.0	78.0	80.0	82.0	85.0	88.0	25.0
	3176	64.0	66.0	68.0	70.0	72.0	74.0	77.0	78.5	81.0	84.0	87.0	90.0	92.0	28.0
	3179	71.0	73.0	75.0	77.0	79.0	82.0	85.0	87.0	90.0	88.0	85.0	86.0	88.0	17.0
	3184	79.0	82.0	84.0	88.0	90.0	93.0	96.0	99.0	101.0	104.0	106.0	108.0	110.0	31.0

contd.....

contd..... Table A8

Treatment group	Buffalo calf No.	Initial body weight (kg)	WEEKS												Weight gain (kg)
			1	2	3	4	5	6	7	8	9	10	11	12	
Group 3 (0.6 ppm Af)	3154	61.0	64.0	68.0	72.0	73.0	75.0	77.0	79.0	81.0	82.0	82.0	79.0	80.0	19.0
	3165	72.0	74.0	75.0	77.5	80.0	82.5	84.0	86.0	84.0	84.0	87.0	88.0	91.0	19.0
	3172	46.5	48.0	50.0	51.0	52.0	53.0	53.0	54.0	55.0	56.0	58.0	59.0	60.0	13.5
	3178	77.0	80.0	82.5	85.0	88.0	90.0	93.0	95.0	98.0	101.0	104.0	106.0	107.0	30.0
	3189	55.5	57.0	59.0	60.0	62.0	64.0	66.0	67.0	68.0	71.0	72.0	73.0	75.0	19.5
	3237	61.5	63.0	65.0	66.0	68.0	69.0	70.0	71.0	72.0	72.5	73.0	74.0	74.0	12.5
Group 4 (1.0 ppm Af)	3213	45.0	46.0	46.0	48.5	51.0	54.0	55.0	54.0	56.0	58.0	58.0	59.5	62.0	17.0
	3238	58.0	60.0	62.0	63.0	65.0	66.0	67.5	68.0	69.0	70.0	71.5	72.0	74.0	16.0
	3244	62.0	63.0	64.0	65.0	67.0	69.0	70.0	71.0	72.0	74.0	76.0	78.0	79.0	17.0
	3245	57.5	59.0	60.0	61.0	62.0	62.5	64.0	65.0	66.0	67.0	68.0	70.0	72.0	14.5
	3246	63.0	65.0	66.0	68.0	70.0	72.0	74.0	76.0	77.0	78.0	80.0	82.0	83.0	20.0

Table A9. Digestibility coefficient of nutrient in lactating goats

Treatment group	Goat No.	Digestibility coefficient					
		DM	OM	CP	CF	EE	NFE
Group 1 (0 ppm Af) Control	SB 268	71.78	74.08	70.16	67.20	86.12	76.57
	ASB 355	74.66	75.69	74.27	69.01	80.22	77.76
	ASB 361	75.09	77.64	78.10	68.40	86.22	79.83
	AB 609	68.53	71.14	71.46	72.40	64.00	77.03
	AB 672	69.63	73.25	74.43	60.57	72.84	74.99
	AB 680	78.91	79.99	79.40	74.12	88.63	81.56
Average ± S.E.		73.10±1.58	75.30±1.30	74.64±1.47	68.62±1.93	79.67±3.90	77.96±0.97
Group 2 (1.0 ppm Af)	SB 291	75.76	78.42	78.32	66.67	75.54	81.50
	ASB 369	73.87	77.95	76.08	73.89	80.49	79.49
	ASB 381	72.83	75.65	74.83	68.36	77.46	77.94
	ASB 392	70.08	71.37	74.35	63.78	61.78	73.25
	AB 657	78.70	81.35	81.00	66.32	89.19	84.52
	AB 677	72.44	74.91	79.11	69.38	79.84	75.57
Average ± S.E.		73.95±1.22	76.60±1.40	77.38±1.02	68.06±1.40	77.38±3.66	78.70±1.66

Table A10. Nitrogen balance in lactating goats

Treatment group	Goat No.	N intake (g/d)	N excreted in faeces (g/d)	N excreted in urine (g/d)	N excreted in milk (g/d)	N retained (g/d)	N digestibility (%)
Group 1 (0 ppm Af) Control	SB 268	27.08	8.08	9.84	5.02	4.14	70.16
	ASB 355	29.85	7.68	9.32	4.78	8.07	74.27
	ASB 361	28.50	6.24	8.80	3.96	9.50	78.10
	AB 609	17.10	4.88	5.96	2.90	3.36	71.46
	AB 672	25.69	6.57	6.27	3.98	8.87	74.43
	AB 680	29.48	6.08	6.80	4.76	11.84	79.40
Average ± S.E.		26.28±1.94	6.59±0.47	7.83±0.69	4.23±0.32	7.63±1.33	74.64±1.47
Group 2 (1.0 ppm Af)	SB 291	13.10	2.84	6.84	2.04	1.38	78.32
	ASB 369	20.07	4.80	9.36	4.30	1.61	76.08
	ASB 381	17.80	4.46	6.28	3.68	3.36	74.83
	ASB 392	22.36	5.60	8.64	2.28	5.84	74.95
	AB 657	21.05	4.00	9.84	3.02	4.19	81.00
	AB 677	15.51	3.24	6.40	3.48	2.39	79.11
Average ± S.E.		18.32±1.44	4.16±0.42	8.56±0.76	3.13±0.35	3.13±0.69	77.38±1.02