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CHANGES IN AMINOACIDS, UREA NITROGEN AND URIC ACID IN CHICKENS SERA DUE TO MYCOPLASMA GALLISEPTICUM INFECTION

BY

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INTRODUCTION

Economic losses from *M. gallisepticum* infection resulting from reduced weight gain, feed effeciency, decreased egg production, increased condemnation and medication cost (Yoder 1985) increased the demand for more study about this organism.

One of the important steps for combating any disease is to study the properties of the causative organism, its mode of action and its effect on the host which lead to effecient methods of diagnosis, treatment and control (Williams and Nunn, 1978). The biochemistry and the biochemical methods play a very important role in realizing this purpose and that is why the biochemical analysis are considered the first line of defence against the spread of infection within the laboratory (Levine and Becker, 1977).

Serum free amino acids of chickens infected with respiratory diseases including Mycoplasma were studied

by many authors e.g. Cole and Boyd (1965), Kludas (1968), Squibb and Reed (1969), Wanne Marcher et al., (1972); Blackburn (1978), Ivanov and Doicheva(1979) and Maurice et al., (1983). They concluded that the degree of changes in free amino acids, urea nitrogen and uric acid characterizes the direction of pathogenic and metabolic process in infected chicks.

The aim of this work was to study some biochemical constituents of the serum of chickens infected with *M. gallisepticum* . e.g. urea introgen, uric acid and amino acid pattern.

MATERIALS AND METHODS

Samples:

1. Birds: fifty, day old Hubbard chicks were brought from General Poultry Company. The birds were apparently healthy and serologically negative for Mycoplasma, no medication was previously administered to them and the maintenance and feeding during the period of the experiment were the same till they became 2 weeks old.

2. Blood: blood samples were colected from infected and control chickens after one and four weeks of infections.

3. Tissue: Fresh parts of lungs, tracheas and air sacs were taken from slaughtered chickens.

An experimental infection was adopted by dividing the birds into 4 groups, each of 12 chickens, according to the route of infection with 0.2 ml 24-36 hrs broth culture of *M. gallisepticum* strain PG 31 after several passage in egg yolk without thallium acetate, 2×10^{-8} colony form. unit (C.F.U.), according to the method of inoculation described by Kuba et al., (1974) group 1 was inoculated intransally, group 2 was inoculated intratracheally, group 3

via air sac and group 4 was considered as, control.

The period of the experiment was one month during which the samples were examined at least in the 1st and 4th week of infection.

Blood samples were submitted to the following serologic examination:

1. Slide Agglutination test (Adler et al., 1958)

2. Growth inhibition test (Clyde, 1964) using *M. gallisepticum* stained antigen, supplied by Salsbury laboratories U.S.A.

Tissue samples were cultivated on heart infusion media (Hayflick, 1965) as described by El-Ebeedy (1973). The reisolated isolates were examined by Digitonin test for genus determination as described by Freund (1973). Biochemical characterization was done according to Sabry (1968).

Identification was carried out using growth inhibition test (Clyde, 1964). *M. gallisepticum* type culture and antisera were obtained from National Institute of Allergy. Bethesda, Mayland, U.S.A.

Biochemical analysis:

The serum was used for the determination of:

1. Serum urea nitrogen
2. Serum uric acid.
3. Amino acid pattern

Serum urea nitrogen: was determined using Auto-Analyzer (Marsh, 1965) utilizing thiosemi-carbazide in acid solution, diacetyl monoxime is hydrolyzed to diacetyl and react with urea by a condensation reaction. The samples were diluted with diacetyl monoxime-thiosemicarbazide. The optical density of the mixture was measured at wave length 520 nm.

Serum uric acid was determined using the automated method of Tarry town (1967) by measuring the absorbace of the reaction mixture resulting from the reduction of the phosphotungestate complex at 660 nm.

Serum amino acid pattern was determined using the amino acid Auto-Analyzer technicon model R/20 according to Banson and Patterson (1965).

The sample was introduced to the top of the column, filled with ion exchange resin chromabeads type B provided by Technicon chemistry (U.S.A.) the resin retards the different amino acids for characteristic time till each amino acid was separated and determined using the density of the blue colour with ninhydrine colouring reagent using colorimeter at 570 nm and their values were plotted against time on a moving strip chart. The absorption of proline was measured at 440 nm.

Statistical analysis was done according to Turner (1970), Cross Land (1971) and Goldstein (1965).

RESULTS

Gross lesions were mild showing slight congestion in the lungs, slight turbidity in the air sacs, frocy exudate in the trachea and slight liver congestion and perihepatitis in infected chickens.

Serological examination:

1. Slide agglutination test (SAT):

The number of poitive sera and the intensity of agglutination increased in the 1st week of infection, (+++) in case of infection via air sac and intratacheally, and (++) in intransally infected ones then decreased graudally whatever was the route of infection.

Table (1 a,b): Statistical analysis of total serum urea nitrogen (SUN) and serum uric acid (SUA) in sera groups of control and infected chickens at the 4 weeks of infection.

a) Control group

Statistical parameters		SUN (m mol/L) 1st week	SUN (m mol/L) 4th weeks	SUA (m mol/L) 1st week	SUA (m mol/L) 4th weeks
Arith. mean ($\bar{x}$) ± S.E.		(2.856±0.092)	(2.737±0.151)	(0.426±0.013)	(0.474±0.021)
Measurs of individual variability	Range	(2.499-3.213)	(2.142-3.213)	(0.369-0.458)	(0.375-0.518)
	S.D.	0.226	0.369	0.032	0.051
	C.V. %	7.91%	13.47%	7.44%	10.67%
95% confidence limit of universal mean		(2.619-3.093)	(2.350-3.124)	(0.393-0.459)	(0.421-0.527)
Expected limits of the normal range of individual estimates		(2.528-3.184)	(2.202-3.272)	(0.380-0.472)	(0.401-0.547)

2. Growth inhibition test (GIT):

The test was negative in the 1st week of infection while growth inhibiting antibodies were detected in the 4th week. The widest inhibition zones were seen in case of chickens infected via air sac followed by intratracheal and then intranasal.

The reisolation rate of *M. gallisepticum* from infected birds was very low (17%-33%) in case of chickens inoculated intratracheally and 17% in chickens infected through air sac and intranasally while there was significant increase ($p < 0.001$) in chickens intratracheally infected (after one week).

Regarding the biochemical results, serum urea nitrogen (SUN) and serum uric acid (SUA)(mmol/L) were statistically presented in Table, (1a, b) and illustrated in Fig. 1.

SUN was significantly decreased in infected chickens compared with normal control values as in Table 1a. In the 1st week in case of chickens infected via airsacs the SUN was 2.213 ± 0.071 while in the control group it was 2.856 i.e. there was significant deviation from normal.

From Fig. 1 it was clear that the mean % of deviation (decrease) from normal range differs according to the period and route of infection. e.g. it was 17% in case of chickens infected via air sac in the 1st week of infection and 2.5% in the 4th week while it was 8% and 17% in the 1st and 4th week of infection, respectively, in intranasally infected chickens.

Regarding SUA level it was concluded from the data presented in Tables 1a, b to be significantly increased in *M. gallisepticum infection* during the experiment whatever was the route of infection, e.g., it was found that SUA level was 0.426 ± 0.13 in control

Table (1) b. Infected groups

Serum urea nitrogen & uric acid		% of incidence frequences among 6 infected chic infected via : $\bar{x} \pm$ S.E. , (Range in brackets) for estimates.		
		intra air sac route	intra nasal route	intra trach
urea nitrogen m mol/L after one week	i	in 17% (1/6) 2.856 .	in 50% (3/6) 3.094 + 0.075 (2.856 - 3.213)	in 67% (4/6) 3.213 + 0 (3.213 in each chicks
	ii	in 83% (5/6) 2.213± 0.071 (2.142 - 2.499) -16%*	in 50% (3/6) 2.380 ± 0.075 (2.142 - 2.499) -9%*	in 33% (2/6) average 2.142 (2.142 in each ckicks -15%*
urea nitrogen m mol/L after 4 weeks	i	in 50% (3/6) 2.18 ± 0.075 (2.499 - 2.856)	in 67% (4/6) 2.678 ± 0.080 (2.499 - 2.856)	in 83% (5/6) 2.606 ± 0.156 (2.321 - 3.213)
	ii	in 50% (3/6) 2.142 ± 0 (2.142 in each chick) -3%*	in 33% (2/6) average 1.9635 (1.785 - 2.142) -16%*	in 17% (1/6) 2.142 . -9%
Uric acid m mol/L after one week	i	in 33% (2/6) average 0.455 (0.440 - 0.470)	—	—
	ii	in 67% (4/16) 0.484 ± 0.003) +5%* (0.476-0.488)	in 100% (6/6) 0.644 ± 0.016 (0.595 - 0.690) +36%*	in 100% (6/6) 0.579 ± 0.006 (0.565 - 0.595) +26%*
Uric acid m mol/L after 4 weeks	i	in 67% (4/16) 0.442 ± 0.008 (0.434 - 0.008)	in 17% (1/6) 0.402 .	in 33% (2/6) average 0.536 (0.530 - 0.542)
	ii	in 33% (2/6) average 0.551 (0.548 - 0.553) +4%*	in 83% (5/6)_ 0.594 ± 0.011 (0.559 - 0.631) +13%*	in 67% (4/6) 0.635 ± 0.028 (0.595 - 0.714) +20%*

i = within the normal range

ii = significantly deviated from normal

chickens while it was 0.579 ± 0.006 in intratracheally infected group.

From Fig. 1 it was clear that there was significant increase in the mean % deviations from the normal range especially in chickens infected intranasally in the 1st week of infection (36%).

As for serum aminoacid pattern Tables 2-4 and Fig.2 showed that there was significant decrease in serum amino acid levels through the period of the experiment whatever the route of infection was e.g. Total amino acid was 534 in the control group while it was 289, 339, 372 in chickens infected via air sac, intratracheally and intranasally after one week infection. After 4 weeks of infection the total amino acids was 279, 311, 360 in chickens infected via air sacs, intratracheally and intranasally respectively but it was 479 in the control group as shown in Table 3. Fig. 2 showed a clear mean % deviation of serum aminoacids from normal range which is very prominant in case of infection via air sac a week after infection.

DISCUSSION

The changes of biochemical constituents of chickens blood due to *Mycoplasma gallisepticum* infection had been studied by many authors allover the world e.g. Clyde and Thomas (1973) and Austic and Cole (1977).

In Egypt, Yousef (1972) spotted the light on some biochemical changes due to Mycoplasma infection in chickens but he did not mention the species of Mycoplasma included. El-Shabiny (1984) studied serum protein and enzymatic changes in association with *M. gallisepticum* infection.

The present study was carried out to gain more informations about the altration of biochemical constituents in chickens sera in association with *M. gallisepticum* infection. An experimental infection with

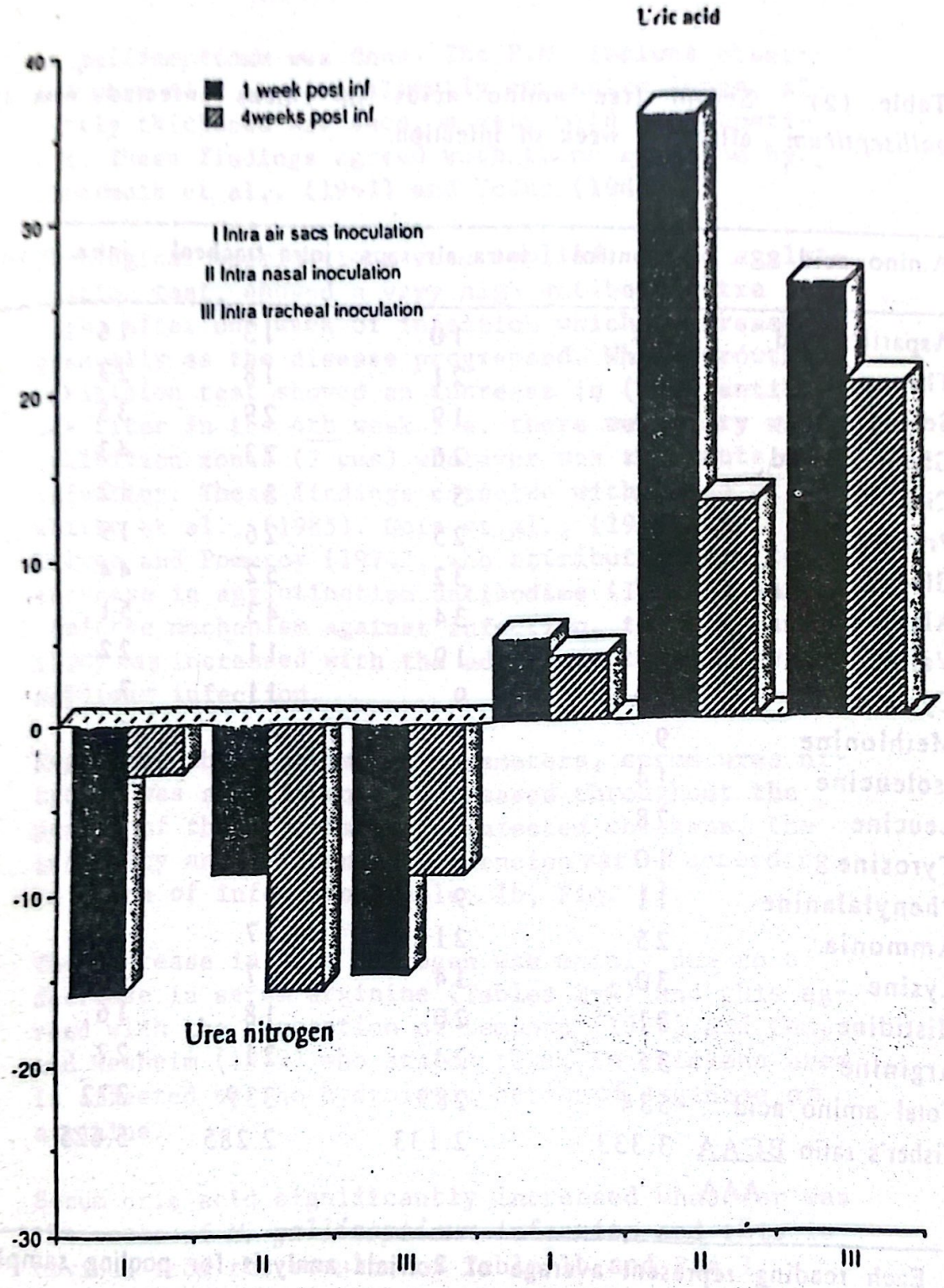

Fig. 1 : Mean % deviations from the conventional limits of the normal range for urea nitrogen and uric acid in *M. gallisepticum* infected chickens.

Table (2) Serum free amino acids in chicks infected with *gallisepticum* after one week of infection.

Amino acid	Control	intra air sacs	intra tracheal	intra nasal
Aspartic acid	14	10	13	16
Threonine	54	21	18	23
Serine	65	19	29	35
Glutamic acid	57	26	23	43
Citrulline	14	5	8	12
Proline	27	25	26	19
Glycine	46	32	52	44
Alanine	61	34	43	51
Valine	28	19	11	22
Cysteine	10	9	11	7
Methionine	9	3	4	6
Isoleucine	14	4	9	7
Leucine	28	9	12	16
Tyrosine	10	6	8	4
Phenylalanine	11	9	6	4
Ammonia	25	21	27	19
Lysine	30	14	27	20
Histidine	21	20	18	16
Arginine	35	24	21	27
Total amino acid	534	289	339	372
Fisher's ratio $\frac{BCAA}{AAA}$	3.333	2.133	2.285	5.625

* Each reading represent average of 2 trials analysis for pooling samples of 6 chicks individual serum samples.

M. gallisepticum was done. The P.M. lesions observed were mild showing slightly congested lungs, slightly thickened air sacs, a very mild perihepatitis. These findings agreed with those reported by Domermuth et al., (1967) and Yoder (1985).

Serological examinations were applied. Slide agglutination test, showed a very high antibody titre (IgM) after one week of infection which, decreased gradually as the disease progressed. While growth inhibition test showed an increase in (IgG) antibody titer in the 4th week i.e. there were very wide inhibition zones (2 cms) whatever was the route of infection. These findings coincide with those of Whitby et al., (1985). Gois et al., (1972) and Kleven and Pomeroy (1974), who attributed them to increase in agglutination antibodies (IgM) as early defence mechanism against infection, then IgG antibody was increased with the advancement of *M. gallisepticum* infection.

Regarding the biochemical parameters, serum urea nitrogen was significantly decreased throughout the period of the experiment in infected chickens, the intensity and incidence frequencies varied according to route of infection (Table, 1b, Fig. 1).

The decrease in urea nitrogen was mainly due to a decrease in serum arginine (Tables 2-4) and this agreed with the suggestion of Lemonde (1959) and Chu, and Wesheim (1979) who stated that, in chickens urea is affected by the hydrolytic action of arginase on arginine.

Serum uric acid significantly increased whatever was the route of *M. gallisepticum* infection and this is obvious from the results of Table 1b and Fig.1.

Our results are in agreement with that reported by Clyde and Thomas (1973), Austic and Cole (1977) who attributed the elevation of uric acid to arthritis

Table (3) Serum free amino acids in chicks infected with *M. gollisepticum* after 4 weeks of infection.

Amino acid	Control	intra air sacs	intra tracheal	intra nasal
Aspartic acid	11	5	8	11
Threonine	46	20	25	29
Serine	38	24	30	27
Glutamic acid	45	27	25	31
Citrulline	9	7	11	traces
Proline	30	27	25	29
Glycine	51	28	33	48
Alanine	60	28	37	46
Valine	24	18	19	22
Cysteine	7	5	3	5
Methionine	7	3	4	3
Isoleucine	16	11	7	9
Leucine	30	17	12	16
Tyrosine	11	5	10	7
Phenylalanine	13	4	10	7
Ammonia	29	19	30	25
Lysine	26	19	15	22
Histidine	24	20	17	26
Arginine	31	29	20	22
Total amino acid	479	297	311	360
Fisher's ratio	2.917	5.111	1.9	3.357

* Each reading represent average of 2 trials analysis for pooling samples of 6 chicks individual serum samples.

and synovitis in hocks caused by *M. gallisepticum* infection and also may be due to degradation of antigen antibody complex as well as leucocytes as a result of the active defence mechanism during *M. gallisepticum* infection causing increased protein catabolism.

Harper (1988) reported that uric acid and guanine are the end products of purine and protein catabolism in chickens. On contrary, Yousef (1972) demonstrated a significant decrease in serum uric acid in Nicols chickens infected with mycoplasmosis and attributed such decrease to the chronic wasting course of this disease.

A significant decrease in serum aminoacids was observed from Tables 2-4 and Fig, 2. whatever, was the route of infection. This was in agreement with Yousef (1982), who demonstrated a significant decrease in blood total nitrogen and attributed it to the chronic wasting course of the disease caused by *M. gallisepticum* (CRD) which lowers the appetite and subsequently the weight of infected birds.

Kludas (1968) recommended that amino acids and peptides are required in diet as a source of growth during *M. gallisepticum* infection in chickens.

Many factors were also concerned in the decrease of serum uric acid found in our study such as the formation of new protein for healing dammaged cells and desquamated epithelium (Bell & Siller, 1961) and increased synthesis of immunoglobulins (Makhumuto, 1969 and Petro and Bhattacharijee, 1980).

Hypo-aminocidemia due to the infection may appear even longer before clinical evidence of the disease is apparent (Wannemarcher et al., 1972).

Decreased blood glucose as a result of *M. gallisepticum*

Table (4) : Statistical significance of differences between corresponding amino acids estimates in control and infected chickens with M. gallisepticum

Amino acids groups	Duration of expirment	Routes of infection		
		intra air sacs	intra nasal	intra tracheal
I- Branched chain amino acids (BDAA). (Valine , leucine and isoleucine)	a	N=6 (control) N` = 6 (infected) U value = 4*↓	U = 8↓	U = 0**↓
	b	N=6 (control) N`=6 (infected) U value = 8↓	U = 5*↓	U = 4*↓
II- Aromatic amino acids (AAA) (phenyl alanine , tyrosine)	a	N=4 (control) N` = 4 (infected) U value = 0*↓	U = 0*↓	U = 0*↓
	b	N=4 (control) N` = 4 (infected) U value = 0*↓	U = 0*↓	U = 0*↓
III- non hydroxylated (glycine , alanine) and hydroxylated aliphatic amino acids (Serine , threonine)	a	N=8 (control N` = 4 (infected) U value = 0**↓	U = 0**↓	U = 4**↓
	b	N=8 (control) N` = 8 (infected) U value = 0**↓	U = 5**↓	U = 0**↓
IV Sulphur containing amino acids (cysteine, and methionine	a	N=4 (control) N` = 4 (infected) U value = 1*↓	U = 0*↓	U = 0*↓
	b	N=4 (control) N` = 4 (infected) U value = 0*↓	U = 0*↓	U = 0*↓
V- Acidic amino acids (aspartic , glutamic acids)	a	N=4 (control) N` = 4 (infected) U value = 4↓	U = 8↓	U = 4↓
	b	N=4 (control) N` = 4 (infected) U value = 4↓	U = 5↓	U = 4↓
VI- Basic amino acids (Aginine , lysine , histicline)	a	N=6 (control) N` = 6 (infected) U value = 4*↓	U = 4*↓	U = 5*↓
	b	N=6 (control) N` = 6 (infected) U value = 4*↓	U = 4*↓	U = 5*↓
VII- Urea cycle amino acids (argnine, citrullin).	a	N=4 (control) N` = 4 (infected) U value = 4↓	U = 4↓	U = 4↓
	b	N=4 (control) N` = 4 (infected) U value = 4↓	U = 4↓	U = 4↓
VIII - Imino acid (proline)	a	↓ -7%	↓ -30%	↓ -4%
	b	↓ -10%	↓ -3%	↓ -17%

(Wiloxon two samples rank test or Mann. Whitney U test).

a) after one week of infection
b) after four weeks of infection
N = Numbers of control samples
N` = Numbers of infected samples
* Significant P< 0.05 , 0.025
** Highly significant P<0.005

infection as was reported by Longslow et al., (1970) and Siegel et al., (1972) may stimulate gluconeogenesis which subsequently lowers the level of free amino acids.

The perihepatitis due to *M. gallisepticum* infection in chickens (Ferenci, 1986), could be responsible for impaired glucose oxidation that stimulated gluconeogenesis and resuction of branched chain amino-acid levels.

Ross et al., (1955), Makhumuto (1969) and Petro and Bhattacharijee (1980) demonstrated significant decrease of methionine in sera of chickens infected with *Salmonella pullorum* and that was due to requirement for methionine for antibody formation.

The typical changes of serum aminoacids in liver function described by the molar ration is correlated with branched chain/aromatic amino acids BCAA: AAA (Fisher's ratio) as it is clear from Table 2,3,4 and Fig. 2.

In the present study, the decrease in Moler Figher's ratio in sera of chickens after one week of infection via air sacs and intratracheally and those after four weeks of infection intratracheally, indicates poor hepatocellular function, while the increased Fischer's ratio in intratranasally infected chickens during the period of the experiment, and those after weeks of infection via air sac may be due to a more pronounced decrease in aromatic amino acids which is indicative of permenant dammage of liver tissue, (Kraczkowski 1964 and Ferenci 1986).

The control values of the serum urea nitrogen in our study are in agreement with those reported by Solima et al., (1966) and Koudela et al., (1977).

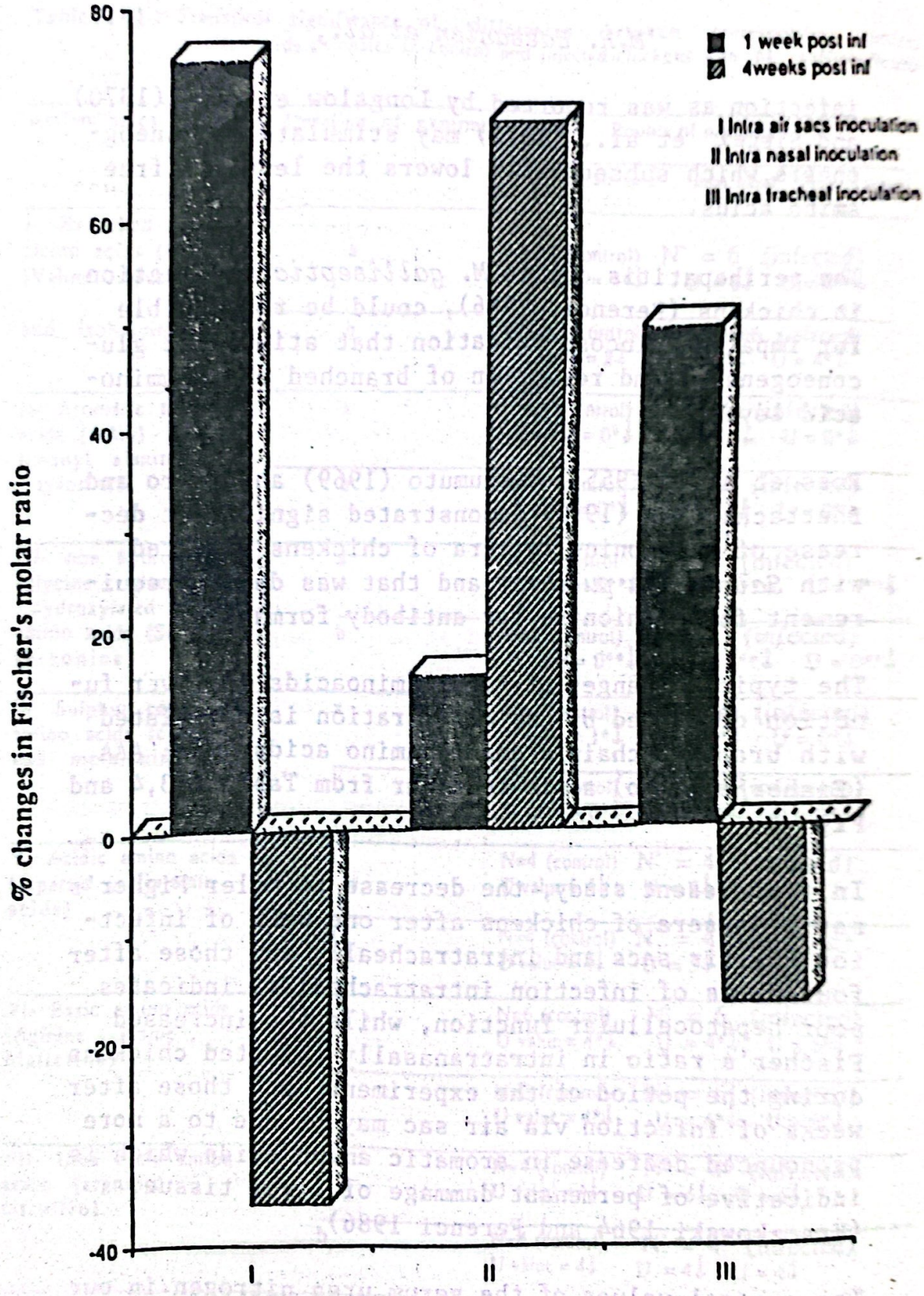

Fig 2 : Mean % deviations from the conventional limits of the normal range for Fischer's molar ratio BCAA/AAA in *M. gallisepticum* infected chickens.

Considering serum uric acid, our results of control values coincide with those reported by Ross et al., (1976), Kiefer (1979) and Koudela and Roubal (1982), Regarding serum aminoacids, our results are similar to those found by Cole and Boyd (1965), Taylor et al., (1970), Desmarais and Pare (1972) and Maruyama et al., (1976).

Slight fluctuation in such values might be due to individual peculiarities of the bird.

SUMMARY

The present study was conducted to investigate the altration of some biochemical parameters in sera of chickens i.e. amino acids, urea nitrogen and uric acid in association with *M. gallisepticum* infection.

The target population was 50,2 weeks old chicks from General Poultry Company. They were devided into 4 groups, 12 chicks each. They were experimentally infected with *M. gallisepticum* via different routes (intra-air sac, intranasal and intra-tracheal). A control normal group of birds was considered. The chicks were examined after one week and 4 weeks of infection.

The reisolation rate was low in all infected chicks whatever was the route of infection.

The maccopathological lesions were very mild i.e. slightly congested lungs, slightly thickened air sacs and the liver with slight perihepatitis. Those lesions increased in degree with advancement of infection.

Serological examination showed high titre of agglutinating antibodies at the beginning of infection then decreased gradually till the 4th week. On the contrary,

the growth inhibiting antibodies began at the 2nd week of infection and increased gradually during the period of the experiment.

The estimation of the biochemical parameters in sera of infected chickens revealed significant decrease in urea nitrogen during the period of infection whatever was the route of infection while the serum uric acid showed significant increase in levels, incidence and frequency in the early stage of infection than later on towards the end of the experiment.

The serum amino acid pattern obtained by Auto-Analizer showed a decrease in all individual amino acids and a significant decrease in non hydroxylated and hydroxylated amino acids (glycin, alanine, serine and threonine). While acidic, urea cycle amino acids (Arginine and Citrullin and proline) were insignificantly decreased.

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