EFFICACY OF DIFFERENT METHIONINE SOURCES FOR BROILERS

By

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B.V.Sc. & A.H.

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COLLEGE OF VETERINARY SCIENCE
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RAJENDRANAGAR, HYDERABAD - 500 030

1997

CERTIFICATE

Mr.N.RAVI PRAKASH REDDY has satisfactorily prosecuted the course of research and that the thesis entitled **EFFICACY OF DIFFERENT METHIONINE SOURCES FOR BROILERS** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that, the thesis or part thereof has not been previously submitted by him for a degree of any university.

Date: 16.9.97

(Dr.V.RAMA SUBBA REDDY)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **EFFICACY OF DIFFERENT METHIONINE SOURCES FOR BROILERS** submitted in partial fulfilment of the requirements for the degree oif **MASTER OF VETERINARY SCIENCE** of the ANGRAU, Hyderabad is a record of the bonafied research work carried out by **Mr.N.RAVI PRAKASH REDDY** under my guidence and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree (or) diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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(N.RAVI PRAKASH REDDY)

DECLARATION

I, **N.RAVI PRAKASH REDDY** hereby declare that the thesis entitled **EFFICACY OF DIFFERENT METHIONINE SOURCES FOR BROILERS** submitted to the Acharya N.G.Ranga Agricultural University for the degree of **MASTER OF VETERINARY SCIENCE** is a result of the original research work done by me. I also declare that the thesis or any part thereof has not been published earlier elsewhere in any manner.

Date:

Place: Hyderabad.

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Year of submission	:	**1997**

ABSTRACT

Two experiments were conducted to evaluate the dietary requirement of total sulphur amino acids (TSAA) and efficacy of Alimet(R) (MHA-FA) as methionine source in commercial male broilers maintained in battery brooders. In both the experiments chicks were fed on diet containing 192 g/kg CP and 9 g/kg TSAA during pre-experimental period. The feed ingredients and experimental diets were analysed for total amino acids by M/s. Degussa. In experiment 1, the corn-soyabean basal diet, containing 6 g/kg TSAA (3 g/kg methionine) was supplemented with graded levels of DL-methionine at 6.0, 6.6, 7.2, 7.8, 8.4, 9.0, 9.6 and 1.02 g/kg diet of TSAA. On each diet, 5 groups of 5 broilers each were fed from 8-21 days. In experiment 2, a corn-soyabean, sorghum and peanut meal basal diet containing 6.2 g/kg TSAA (3.2 g/kg methionine) was supplemented with DL-methionine at 1.32 g/kg and Alimet at 2.0 g and 1.47 g/kg diet. Assuming an efficacy of 65 per cent and 88 per cent, Alimet at 2.0 g and 1.47 g respectively was equivalent to 1.3 g DL-methionine. 10 groups of 10 broilers per group were placed on each diet. The diets were fed from 9-28 days. In both the experiments weight gains, feed intake and feed/gain data was analysed for analysis of variance.

In experiment 1, supplementation of DL-methionine to broilers resulted in progressively increased weight gains and improved feed efficiency upto 7.2 g/kg TSAA (4.2 g methionine) in the diet. In experiment 2, the performance of chicks on diets containing supplemental DL-

methionine (1.3 g/kg) and Alimet at an assumed activity of 65 per cent (2 g/kg) were statistically similar and significantly ($P<0.05$) better than on reference diet and the diet containing Alimet at 1.47 g/kg.

The livability on all the diets was similar. Mortality was with in the range and not related to dietary variation in TSAA and methionine.

It may be inferred that the requirement of TSAA may be 7.2 g/kg (methionine representing 58.3% of TSAA) for broilers (8.21 days age). Alimet(R) appears to have an efficacy of 65 per cent rather than 88 per cent based on the performance of broilers.

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LIST OF ABBREVIATIONS

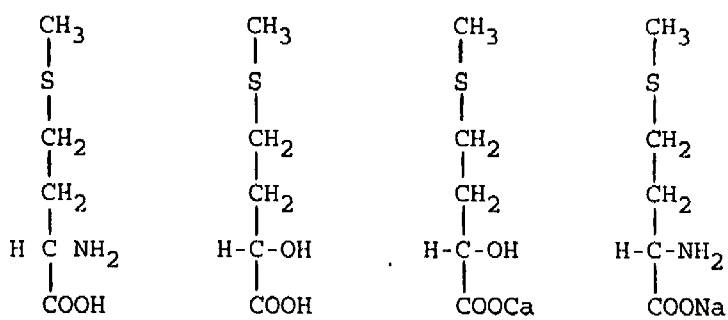
b	:	birds
CP	:	crude protein
d	:	day(s)
DLM	:	DL-methionine
FE	:	Feed efficiency
g	:	grams
kcal	:	kilocalories
kg	:	kilograms
mg	:	milligrams
MHA-Ca	:	Methionine hydroxy analogue-calcium
MHA-FA	:	Methionine hydroxy analogue-free acid
%	:	per cent
TSAA	:	Total sulphur amino acids

INTRODUCTION

CHAPTER I

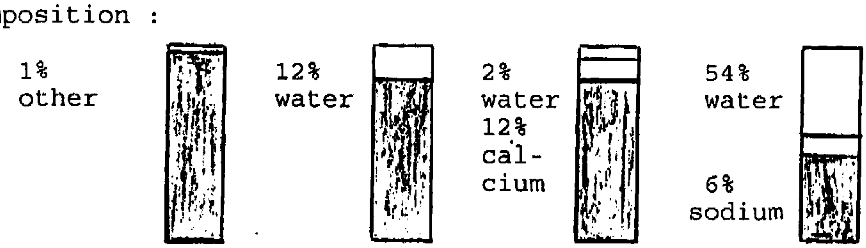
INTRODUCTION

The amino acids, lysine and methionine are the limiting amino acids in broiler feed formulations. In our country, the limited information available is much variable on the requirement of these amino acids (Ramasubba Reddy, 1996). A precise requirement for these amino acids is useful in formulating feeds more efficiently. The following methionine activity products are available for commercial supplementation.



	DL-methionine	MHA-FA(Alimet)(R)	MHA-Ca	DL-sodium methionate
Manufacturing by :	M/s.Degussa	M/s.Novus International	M/s.Novus International	M/s.Degussa
Physical state :	Dry	Liquid	Dry	Liquid

Composition :



Methionine activity %	99	88	86	40

Several workers reported varying methionine efficacy from 67 to 88 per cent for Alimet$^{(R)}$.

The present investigation was taken up with the following objectives.

1. To determine the requirement of DL-methionine in broilers.

2. To evaluate the efficacy of utilization of Alimet$^{(R)}$ (MHA-FA) as a methionine source.

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REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Considerable work has been done in the advanced countries on the amino acid requirements for poultry (NRC, 1994). However, the limited information available on the requirements of the two limiting amino acids for poultry in our country is much variable (Ramasubba Reddy, 1996).

The following review embodies some of the recent reports on the requirement of methionine and total sulphur amino acids (TSAA) and efficacy of methionine hydroxy analogue-free acid (MHA-FA) in relation to DL-methionine for broilers. Also presented in this chapter is the influence of protein, energy, age and the effect of methionine source under heat stress conditions on the performance of broilers.

2.1 REQUIREMENTS OF METHIONINE AND TOTAL SULPHUR AMINO ACIDS

In our country, the very limited information ilable has been recently reviewed (Table 1, Ramasubba Reddy, 1996).

The methionine and TSAA requirement of broilers based on work done in other countries and suggested by different workers is shown in Table 2 and Fig.1 for 1-21 d age, in Table 3 and Fig.2 for 22-42 d age and in Table 4 for 43 d age onwards. Considerable variation exists among the suggested requirement of methionine and TSAA during any phase of growth. Part of the variation may be due to experimental conditions, nutrient levels, bird type and age.

The suggested methionine and TSAA requirement of broilers recommended by different agencies is shown in Table 5. On energy basis, large differences exist on the requirement figures given in Table 5.

Table 1: Published Information in India on the requirements of energy, protein, lysine, methionine and total sulphur amino acids (TSAA) - Broilers

Reference	Brids x group	Exptl period d	Weight gain g/b/d	Feed intake g/b/d	ME kcal/kg	Protein g/kg	Lysine g/kg	Meth-ionine g/kg	TSAA g/kg
						on a diet of 2900, kcal ME/kg			
Desai et *al.* (1975)	B10x2	8-49	m,f 21.3	48	2685	243	10.6	5.2	9.1
Shingari et *al.* (1976)	B10x2	W8-28	m,f 19.7	38					
	10x3	S8-28	m,f 17.3	29	2851	203	9.4	3.9	6.8
	10x2	Sp8-28	m,f 20.6	41					
	10x2	r8-28	m,f 15.0	28					
Ayodhya Prasad et *al.* (1978)	B9x2	8-42	m,f 35.1	82	2713	206	9.2	4.2	7.0
Baghel and Pradan (1988a)	L15x12	1-21		-	2738	210	10.7	5.8	8.9
		22-42	m,f 24.1	-	2770	192	8.7	4.0	6.8
		43-56	-	-	2767	173	7.4	3.0	5.7
Baghel and Pradan (1988b)	L15x2	1-21	-	-	2707	230	10.1	5.1	8.5
		22-42	m,f 27.1	57	2750	225	8.2	3.4	6.5
		43-56	-	-	2761	196	7.1	3.1	6.0
Bhaghel and Pradan (1989)	L15x2	1-21	-	-	2712	212	10.6	5.6	8.6
		22-42	m,f 27.1	59	2761	208	9.1	4.2	7.3
		43-56	-	-	2767	179	7.6	3.1	5.9

B - Battery; L - Litter

Bold faced letters indicate the area of study by the investigator

r - rainy; s - summer; Sp - spring; w - Winter; m - male; f - female

Table 2: Information published on the requirement of methionine and total sulphur amino acids (TSAA) for broilers from 1-21 days age

Reference	Birds/ Experimental period (days)	Crude protein g/kg	Methionine levels tested g/kg	Criterion	Methio-nine g/kg	TSAA g/kg
Nelson et al., 1960	1-28 Rhode Island Red x Plymouth Rock	207	4.8, 5.8, 6.8, 7.8, 8.8, 9.8, 10.8, 11.8, 12.8	FE	6.9	7.6
	1-33 Rhode Island Red x Plymouth Rock	205	3.5, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0	FE	6.4	7.4
Klain et al., 1960	7-14	300	0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0	Growth, FE	1.8	4.7
Dean and Scott, 1965	7-14 New Hampshire x Columbian	250	3.5, 4.5, 5.5 6.5	Growth, FE	4.5	8.0
Bishop and Hallaran, 1967	1-28	-	-	Growth	-	8.3
Graber et al., 1971	8-14	-	3.8, 4.8, 5.3, 5.8, 6.3, 6.8	Growth, FE	-	6.0
Hewitt and Lewis, 1972	7-21	180	2.9, 3.9, 4.4 4.9, 5.9, 6.9	Growth, FE	3.9	7.9

Contd..

Reference	Birds/ Experimental period (days)	Crude protein g/kg	Methionine levels tested g/kg	Criterion	Methio-nine g/kg	TSAA g/kg
Boomgaardt and Baker, 1973b	14-28 New Hampshire x Columbian	230	3.9, 4.6, 5.4, 6.2, 7.0, 7.7, 8.5	Growth, FE	-	7.2 7.3
Woodham and Deans, 1975	14-28	180	5.0, 6.0, 7.0	Growth, FE	-	5.6
Hurwitz et al., 1978	7-14	140	-	-	3.9	8.4
	14-21	140	-	-	3.4	7.8
Willis and Baker, 1980	8-16 New Hampshire x Columbian	-	2.0, 3.0, 4.0, 5.0, 6.0, 7.0	Growth, FE	3.0	6.0
Robbins and Baker, 1980a	8-21 New Hampshire x Columbian	-	1.5, 2.0, 2.5, 3.0, 3.5, 4.0	Growth, FE	2.6	5.2
Robbins and Baker, 1980b	8-21 Hubbard x White Mountain	190	4.3, 4.9, 5.5, 6.1, 6.7	Growth, FE	-	5.5
Willis and Baker, 1981	8-16 New Hampshire x Columbian	-	2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6	Growth, FE	3.3	6.5

Contd..

Reference	Birds/ Experimental period (days)	Crude protein g/kg	Methionine levels tested g/kg	Criterion	Methionine g/kg	TSAA g/kg
Moran, 1981	1-14 White Mountain x Habbard	240	3.6, 4.1, 4.6, 5.1	Growth, FE	4.6	8.7
Wheeler and Latshaw, 1981	1-21 Cobb	202	3.3, 4.3, 5.3, 6.3	Growth, FE	5.9	8.2
Baker *et al.*, 1982	8-18 New Hampshire x Columbian	-	2.0, 2.5, 3.0, 3.5, 4.0, 4.5	Growth, FE	3.0	5.0
Tillman and Pesti, 1985	1-21	216	3.8, 4.1, 4.8, 5.5, 5.8	Growth, FE	5.5	9.1
Hickling *et al.*, 1990	1-21 Ross x Arbor Acres	236	5.0, 5.6	Growth, FE	5.6	9.9
N.R.C. 1994	1-21	230	-	-	5.0	9.0
Schutte and Pack, 1995	14-38 Ross	212	3.3, 3.5, 3.7, 4.0, 4.3, 4.6, 4.9, 5.3	Growth, FE	4.6	8.8

FE - Feed efficiency

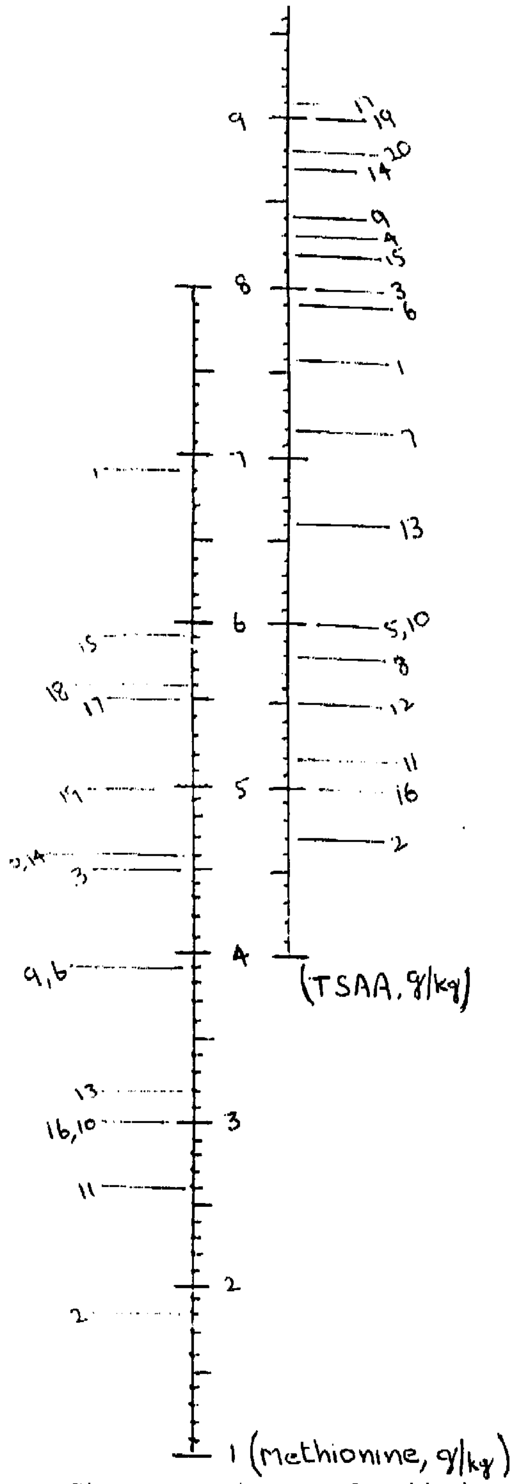


Fig.1: Requirement of methionine and total sulphur amino acids for broilers (1-21d)

References

1. Nelson *et al.* (1960)

2. Klain *et al.* (1960)

3. Dean and Scott (1965)

4. Bishop and Halloran (1967)

5. Graber *et al.* (1971)

6. Hewitt and Lewis (1972)

7. Boomgaardt and Baker (1973b)

8. Woodham and Deans (1975)

9. Hurwitz *et al.* (1978)

10. Willis and Baker (1980)

11. Robbins and Baker (1980a)

12. Robbins and Baker (1980b)

13. Willis and Baker (1981)

14. Moran (1981)

15. Wheeler and Latshaw (1981)

16. Baker *et al.* (1982)

17. Tillman and Pesti (1985)

18. Hickling *et al.* (1990)

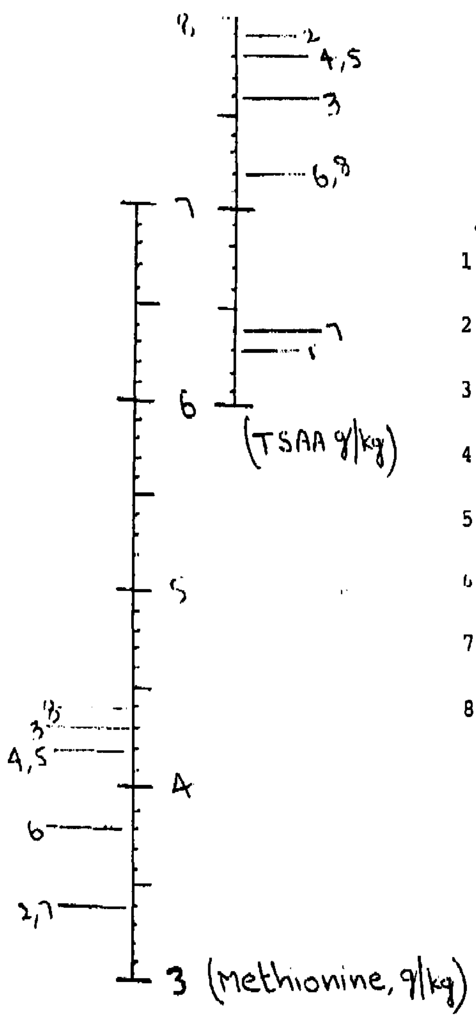
19. N.R.C. (1994)

20. Schutte and Pack (1995)

Table 3: Information published on the requirement of methionine and total sulphur amino acids (TSAA) for broilers from 22-42 days age

Reference	Birds/ Experimental period (days)	Crude protein g/kg	Methionine levels tested g/kg	Criterion	Methionine g/kg	TSAA g/kg
Graber et al., 1971	35-42 New Hampshire x Columbian	-	3.0, 3.5, 4.0, 4.5, 5.0, 5.5 6.0	Growth, FE	-	6.3
Hurwitz et al., 1978	21-28	140	-	Growth, FE	3.4	7.9
	28-35	140	-		3.1	7.6
	35-42	140	-		2.7	6.8
Wheeler and Latshaw, 1981	22-42 Cobb	202	2.8, 3.4, 4.0, 4.6, 5.2, 5.8	Growth, FE	4.3	7.6
Jensen et al., 1989	21-42 Peterson x Arbor Acres	230	3.6, 4.2, 4.8	Growth, FE Carcass fat	4.2	7.8
Hickling et al., 1990	22-42d Rose x Arbor Acres	236	3.8, 4.3	Growth, FE	4.2	7.8
NRC, 1994	22-42	200	-	-	3.8	7.2
Schutte and Pack, 1995	33-43 Cobb	199	3.0, 3.4, 3.9, 4.4, 4.9, 5.5	Growth, FE	3.4	6.4
Baker et al., 1996	22-42	200	2.3, 2.9, 3.5, 4.7, 5.3	Growth, FE	4.4	7.2

FE - Feed efficiency



References

1. Graber *et al.* (1971)

2. Hurwitz *et al.* (1978)

3. Wheeler and Latshaw (1981)

4. Jensen *et al.* (1989)

5. Hickling et al. (1990)

6. N.R.C. (1994)

7. Schutte and Pack (1995)

8. Baker *et al.* (1996)

Fig.2: Requirement of methionine and total sulphur amino acids for broilers (22-42 d)

Table 4: Information published on the requirement of methionine and total sulphur amino acids (TSAA) for broilers from 43 days onwards

Reference	Birds/ Experimental period (days)	Crude protein g/kg	Methionine levels tested g/kg	Criterion	Methio-nine g/kg	TSAA g/kg
Graber *et al.*, 1971	45-56 New Hampshire x Columbian	-	6.5, 7.0, 7.5, 8.0	Growth, FE	6.5	6.5
Boomgaardt and Baker, 1973	42-56 New Hampshire x Columbian	200	3.4, 4.1, 4.8, 5.5, 6.2, 6.9	Growth, FE	5.4 5.6	5.4 5.6
N.R.C., 1994	43 onwards	180	-	-	3.2	6.0

Table 5: Suggested methionine and Total sulphur amino acids (TSAA) requirements of briolers

Reference	Period (days)	ME kcal/kg	Protein g/kg	Lysine g/kg	Methionine g/kg	TSAA g/kg
Ramasubba Reddy, 1996	1-21	2900	220	10.3	5.4	8.8
	22-42	2900	200	8.9	4.0	6.8
	43.56	2900	180	7.5	3.1	5.9
Degussa, 1993	1-21	3150	210	12.4	5.6	9.6
	22-49	3200	200	11.2	5.2	9.2
	49 onwards	3250	180	9.8	4.3	8.2
BIS, 1994	Starter	2800	230	12.0	5.0	9.0
	Finisher	2900	200	10.0	3.5	7.0
ICAR, 1985	Starter	2900	240	12.2	4.4	8.3
	Finisher	2900	190	10.6	3.8	7.2
NRC, 1994	1-21	3200	230	11.0	5.0	9.0
	28-42	3200	200	10.0	3.8	7.2
	43 onwards	3200	180	8.5	3.2	6.0

BIS - Bureau of Indian Standards
ICAR - Indian Council of Agricultural Research

2.2 EFFICACY OF METHIONINE HYDROXY ANALOGUE-FREE ACID (MHA-FA) IN RELATION TO DL-METHIONINE IN BROILERS

Three supplements are commonly used to meet the sulphur amino acid requirements in poultry : DL-methionine hydroxy analogue-calcium and DL-methionine as dry powders and methionine hydroxy analogue-free acid as liquid.

Several researchers (Waldroup *et al.*, 1981; Elkin and Hester, 1983; Knight and Giesen, 1983; Schisla *et al.*, 1983; Garlich, 1985) stated that DL-methionine and MHA-FA were equivalent on equimolar basis in promoting growth of birds. While others (Boebel and Baker, 1982; van Weerden *et al.*, 1982; Van Weerden and Schutte, 1983; Muramatsu *et al.*, 1984; Thomas *et al.*, 1991; Huyghebeart, 1993; Rostagno and Barbosa, 1995) concluded that MHA-FA was biologically less active than DL-methionine on equimolar basis.

The review on the biological efficacy of MHA-FA (Table 6) shows the experimental support for MHA-FA to be 68 to 88% of DL-methionine. Part of this variation may be due to differences in the basal diet used (van Weerden *et al.*, 1982), existance of different polymeric forms in MHA-FA (Dimers 15-19%, higher oligomers 1-3%, Boebel and Baker, 1982), poor sensitivity of the experiment and unsuitable evaluation of the data obtained (Huyghebeart, 1993).

2.3 FACTORS INFLUENCING THE REQUIREMENTS OF METHIONINE/TSAA

Several factors influence the requirement of methionine and TSAA. The important factors are protein and energy content of diet and age of the bird.

DL-methionine in broilers

Reference	Birds/ Experimental period (days)	Methionine levels tested g/kg	Criterion	Relative efficacy of DL-MHA-FA versus DLM (%, equimolar)	Relative efficacy of DL-MHA-FA/Versus DLM (weight basis)* %
Waldroup *et al.*, 1981	0-21	6.8, 7.8 8.3	WG FE	Equivalent Equivalent	
	21-42	6.1, 8.6 7.3	WG FE	Equivalent Equivalent	
Boebel and Baker, 1982	8-16 New Hampshire x Columbian	1.0, 2.0 3.0, 4.0	WG FE	78 78	-
	8-15	13.0,15.0	WG FE	80.8 80.8	
van Weerden *et al.*, 1982	6-27 Shaver starbro	4.0, 5.0, 6.0	WG FE	70 71	66
van Weerden and Schutte, 1983	1-38d Shaver starbro	7.0, 7.5, 8.0	WG FE	78 68	63
Elkin and Hester, 1983	1-21 Hubbard	6.6, 7.4	WG FE	Equivalent Equivalent	88
Knight and Giesen, 1983	1-21 Broilers	5.4, 6.2, 7.0 8.6	WG FE	Equivalent Equivalent	
	22-42	4.9, 5.4, 5.9, 7.0	WG FE	Equivalent Equivalent	88
	43-49	4.2, 4.6, 5.1, 6.0	WG FE	Equivalent Equivalent	

Contd..

Reference	Birds/ Experimental period (days)	Methionine levels tested g/kg	Criterion	Relative efficacy of DL-MHA-FA versus DLM (%, equimolar)	Relative efficacy of DL-MHA-FA/Versus DLM (weight basis)* %
Schisla et al., 1983	1-49 Broilers	-	WG FE	Equivalent Equivalent	88
Muramatsu et al., 1984	8-16 White Leghorn chicks	1.0, 2.0, 3.0	WG FE	70 70	-
Garlich 1985	0-21 Peterson x Arbor Acres	6.5, 7.2 7.9, 8.5	WG FE	Equivalent Equivalent	88
	21-42	5.8, 6.3, 6.9, 7.4	WG FE	Equivalent Equivalent	
Balnave and Oliva, 1990	21-42	4.9, 5.3, 5.7, 6.5	WG FE	Equivalent 68	88
Thomas et al., 1991	7-21	6.2, 6.6, 7.0, 7.4, 7.8, 8.2 8.6, 9.0	WG FE	72 72	64
		5.4, 6.0, 6.6, 7.2, 7.8, 8.4, 9.0, 9.6	WG FE	85 78	
Huyghebeart, 1993	8-24	5.7, 6.0, 6.4, 6.8, 7.2, 7.9, 8.2	WG FE	73 68	62
Rostagno and Barbosa, 1995	21-42 Hubbard	5.2, 5.5, 5.8, 6.1	WG FE	83 67	67

WG - Weight gain DLM - DL-methionine

FE - Feed efficiency DLMHA-FA - DL-methionine hydroxy analogue-free acid

D - Days; * Considering the concentration of 88% MHA-FA in the commercial Alimet

2.3.1 Effect of protein on methionine/TSAA requirement

Almquist (1952) reported that methionine requirement increased as the protein level in the diet increased and vice versa and this view was later supported by others (Baldini and Rosenberg 1955, Rosenberg and Baldini 1957, Nelson *et al.* 1960). This relationship has been confirmed for several amino acids including tryptophan (Boomgaardt and Baker, 1971), lysine (Boomgaardt and Baker, 1973a) and threonine (Robbins, 1987).

Nelson *et al.* (1960) reported that the requirement for methionine plus cystine was a constant proportion of the protein in diets ranging from 212 to 275 g CP/kg. Mendonca and Jensen (1989) reported that the requirement for methionine plus cystine increased as the dietary protein concentration was raised beyond 200 g CP/kg.

Morris *et al.* (1992) calculated the methionine concentration in diets to be 0.025 times of the dietary crude protein concentration.

2.3.2 Effect of energy on methionine requirement

Methionine requirement is known to be directly proportional to the energy content of the diet. The amino acid consumption was directly related to feed consumption which inturn is regulated by energy content of the diet (Baldini and Rosenberg, 1955).

The requirement of methionine in isonitrogenous diets, increased as the energy content of the diet was increased (Rosenberg and Baldini, 1957). Increasing dietary metabolisable energy (ME) at particular amino aicd : ME ratios significantly improved growth and food utilization of broilers kept at moderate (18 to 26°C) and high (25 to 35°C) ambient temperatures during finishing period from 22 d of age and the optimum amino acid : ME ratio varied with dietary ME concentration in the hot, but not in the moderate environment (Sinurat and Balnave, 1985).

2.3.3 Effect of age on methionine/TSAA requirement

A review of the effect of age on TSAA requirement in broilers is summarized in table 2, 3 and 4.

Several reports (Nelson *et al.*, 1960; Hurwitz *et al.*, 1978; Wheeler and Latshaw, 1981; Hickling *et al.*, 1990; Schutte and Pack, 1995) indicated that the methionine requirement expressed as a percentage of diet decreases with increasing age.

Graber *et al.* (1971) reported that the methionine requirement (TSAA) slightly increases with advancing age of broilers, but Boomgaardt and Baker (1973b) found that requirement for TSAA decreased per unit of protein in older chicks compared to younger chicks.

2.4 HEAT STRESS

Heat stress due to high environmental temperature adversely affects feed consumption (20%), hence nutrient density should be kept high in heat stress (Ichhponani, 1996). Balnave and Oliva (1990) reported that the methionine requirement for growth was reduced in broilers kept at a constant temperature at 30°C (0.22 g/MJ of ME) compared with a constant 21°C (0.29 g/MJ of ME) and the requirement in a diurnally fluctuating temperature regimen of 25-35°C was intermediate (0.26 g/MJ of ME).

Swick *et al.* (1990) concluded that feeding of broilers upto 6 weeks with Alimet(R) improved the feed intake and livability during heat stress conditions compared to DL-methionine on equimolar basis.

Teeter (1994) found no considerable difference with DL-methionine or DL-MHA-FA in heat stress. Rostagno and Barbosa (1995) reported efficacy of MHA-FA relative to DL-methionine as 83 per cent for weight gain and 67 per cent for food conversion in chickens as affected by heat stress.

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MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

Two experiments were conducted to estimate the requirement of total sulphur amino acids (TSAA) and to determine the efficacy of methionine hydroxy analogue-free acid (MHA-FA) in relation to DL-methionine for broilers. Experiment 1 was conducted from February 1996 and the experiment 2 from March 1996.

3.1 ANALYSIS OF FEED INGREDIENTS AND FEEDS

The feed ingredients and feeds were analysed for protein and amino acids by Degusa, Germany.

Amino acid contents of feed ingredients were determined by ion-exchange chromatography after oxidation and hydrolysis (total amino acid content) or after extraction with diluted HCl (supplemental amino acids). Methionine content of MHA-FA was determined by high performance liquid chromatography.

3.2 EXPERIMENTAL DIETS

The diets were based on corn-soyabean meal in experiment 1 and corn-soyabean meal, peanut meal and sorghum in experiment 2 (Table 7). Methionine and cystine content of the diets was varied by supplemental methionine source to basal diets formulated based on the analysed methionine and cystine values of feed ingredients.

3.3 BIRDS MANAGEMENT

Male broilers were raised on wire floor electrically heated battery brooders. Feed and water were provided *ad libitum*. Broilers were vaccinated against Marek's disease, Newcastle disease and Infectious Bursal disease on 1, 7 and 14 d respectively.

3.4 RESPONSE CRITERION

Weekly body weight of individual bird, group feed intake and mortality during the course of experiments were recorded.

3.5 EXPERIMENT 1: REQUIREMENT OF TSAA FOR BROILERS

The TSAA requirement for commercial broiler males was determined by feeding diets containing graded levels of DL-methionine. Basal diet contained 2870 kcal ME (calculated), 218 g protein, 3.1 g methionine and 6.6 g TSAA (analysed) per kg diet. DL-methionine was supplemented at 0, 0.6, 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 g/kg diet. Each diet was fed to 5 replicates of 5 birds each from 8 to 21 d age. The chicks were fed on diet containing 192 g/kg CP and 9 g/kg TSAA during first seven days of age (Table 7).

3.6 EXPERIMENT 2 : ESTIMATION OF EFFICACY OF MHA-FA IN BROILERS

In experiment 2, the efficacy of MHA-FA as the source of methionine in relation to DL-methionine was tested in broiler diets. Basal diet contained 2935 kcal ME (calculated), 228 g protein, 3.2 g methionine and 6.6 g TSAA (analysed) per kg diet. DL-methionine was supplemented at 0, 1.326 g and MHA-FA at 2.0 g, 1.47 g per kg diet. MHA-FA supplementation at 2.0 g and 1.47 g was equivalent to 1.3 g DL-methionine assuming 65% and 88% activity respectively. Each of these diets was fed to ten replicates of ten birds each from 9 to 28 d age. The chicks were on diet containing 192 g/kg CP and 9 g/kg TSAA during first eight days of age (Table 7).

3.7 STATISTICAL ANALYSIS

The experimental results were subjected to analysis of variance and the differences between the treatment means were compared by their critical difference (Snedecor and Cochran, 1976).

Table 7: Composition of the diets fed to commercial broiler males during pre-experimental period and during experiment 1 (8-21 d) and 2 (9-28 d)

Ingredient	Pre-experimental diet	Experiment 1	Experiment 2
		g/kg	
Yellow corn	661	663	450
Soyabean meal	300	300	260
Sorghum	-	-	150
Peanut meal	-	-	90
Peanut oil	-	-	15
Dicalcium phosphate	17	17	17
Limestone powder	10.9	11	9.5
Common salt	3.5	3.5	3.5
L-lysine HCl	1.6	2.5	2.0
DL-methionine	3	-	-
Trace mineral premix[1]	1	1	1
Vitamin premix[2]	1	1	1
Coccidiostat[3]	1	1	1
Nutrient composition			
Analysed			
Crude protein, g/kg		218	228
Methionine, g/kg		2.5	3.2
Lysine, g/kg		12.6	12.1
Methionine + cystine, g/kg		6.0	6.6
Calculated			
ME, kcal/kg		2870	2935
Crude protein, g/kg		188	203
Methionine, g/kg		3.0	3.0
TSAA, g/kg		6.0	6.2
Lysine, g/kg		1.15	1.13

1. Trace mineral premix provided (mg/kg diet): Zinc, 80; Manganeese, 90; Iron, 60; Copper, 5; Iodine, 0.35.

2. Vitamin premix provided (mg/kg diet); Thiamin, 1; pyridoxine, 2; cyanocobalamine, 0.01, niacin, 1.5; pantothenic acid, 10; tocopherol, 10; riboflavin, 5; retinol acetate, 2.83; cholecalciferol, 0.03; choline, 650.

3. Coban TM (Monensin sodium 10% w/w)

RESULTS

CHAPTER IV

RESULTS

In this chapter, the performance of male broiler chicks fed different levels of DL-methionine and efficacy of Alimet[(R)] in relation to DL-methionine in terms of body weight gains, feed consumption, feed efficiency and livability are presented.

The amino acid composition of feed ingredients is shown in Table 8 and that of experimental diets in Table 9.

4.1 EXPERIMENT 1: DETERMINATION OF TOTAL SULPHUR AMINO ACID (TSAA) REQUIREMENT

TSAA requirement was determined in male commercial broiler chicken by supplementing graded levels of DL-methionine at 0.0, 0.6, 1.2, 1.8, 2.4, 3.0, 3.6 ad 4.2 g/kg to a corn soya based diet providing 2870 kcal ME/kg from 8 to 21 d age (Table 10). The analytical and calculated values for supplemental DL-methionine differed for some diets.

4.1.1 Weight gains

Weight gains of broilers progressively increased with incremental level of supplemental DL-methionine upto 1.2 g/kg. Further increase in the methionine content did not reveal a definite trend.

Analysis of variance showed no signficant differences in the weight gains due to supplemental DL-methionine. This unfortunate and unexpected trend appear to be due to large variation in weight gain data of different replicates. This may be related to mixing of methionine in diets or health status of the birds.

4.1.2 Feed intake

The feed intake of broilers on diets containing varied supplemental levels of DL-methionine did not differ significantly from that at the lowest level of TSAA in diet (6 g/kg).

4.1.3 Feed efficiency

Dietary supplementation of methionine as low as 0.6 g/kg significantly ($P<0.05$) improved the feed efficiency as compared to that on control. Further supplementation however did not produce any improvement in feed efficiency.

Table 8: Analytical amino acid composition of feed ingredients used in experiment 1 and 2 (standardized to a dry matter content of 88%)

Amino acid	Yellow maize		Sorghum		Soyabean meal		Peanut meal	
	Content g/kg	AA in CP %	Content g/kg	AA in CP %	Content g/kg	AA in CP %	Content g/kg	AA in CP %
Methionine	2.0	1.81	1.8	1.91	6.5	1.53	4.3	1.07
Cystine	2.0	1.74	1.8	1.88	6.6	1.55	5.2	1.28
Methionine + cystine	4.0	3.55	3.7	3.80	13.1	3.08	9.5	2.36
Lysine	3.2	2.89	3.5	3.62	26.9	6.32	14.6	3.61
Threonine	3.8	3.37	3.4	3.52	17.7	4.15	11.1	2.75
Tryptophan	0.9	0.76	1.1	1.15	05.8	1.36	4.1	1.02
Arginine	5.0	4.50	4.5	4.69	27.0	6.35	43.4	10.72
Isoleucine	4.2	3.71	3.8	3.96	20.5	4.82	13.2	3.27
Leucine	13.8	12.31	10.7	11.13	34.8	8.17	25.4	6.29
Valine	5.2	4.60	4.8	4.98	21.6	5.08	16.5	4.09
Histidine	3.1	2.72	2.4	2.50	12.4	2.91	9.6	2.39
Phenyl alanine	5.7	5.10	4.7	4.90	23.9	5.60	19.8	4.89
Glycine	4.3	3.86	3.9	3.99	20.1	4.71	23.0	5.69
Serine	5.3	4.71	4.5	4.62	23.3	5.48	19.4	4.79
Proline	9.0	7.99	6.9	7.14	22.5	5.29	16.8	4.16
Alanine	8.1	7.23	7.4	7.70	19.9	4.67	16.0	3.96
Aspartic acid	7.9	7.04	7.6	7.88	52.5	12.34	45.2	11.17
Glutamic acid	20.3	18.06	17.4	18.05	79.2	18.60	70.5	17.42

AA - Amino acid

All amino acids were analysed by the oxidation of the protein hydrolysate except tryptophan which was by Alkaline hydrolysis with barium hydroxide, HPLC determination

1 Analytical Reports from Degussa, 1996

Table 9: Analytical amino acid composition of diets used in experiment I and 2 (standardized to a dry matter content of [illegible]%)

Amino acid[1] g/kg	Experiment I Reference diet	Experiment 2 Reference diet	Reference diet + DL-methionine 1.3 g/kg	Reference diet + Alimet(R) 2 g/kg	Reference diet + Alimet(R) 1.47 g/kg
Methionine	2.5	3.2	4.2	3.1	3.0
Cystine	3.5	3.5	3.4	3.6	3.4
Methionine + cystine	6.0	6.6	7.6	6.6	6.4
Lysine**	12.6	12.1	11.6	11.8	11.1
Threonine	7.9	7.9	7.8	7.8	7.5
Tryptophan	2.3	2.5	-	-	-
Arginine	14.0	16.5	16.1	16.3	15.6
Isoleucine	9.1	9.2	9.3	9.1	8.9
Leucine	19.6	19.4	19.2	19.3	19.0
Valine	10.1	10.2	10.1	10.2	9.9
Histidine	5.8	5.9	5.8	5.9	5.7
Phenyl alanine	10.8	11.2	11.1	11.3	10.9
Glycine	8.9	10.0	9.7	10.1	10.5
Serine	10.6	11.1	10.6	11.1	10.5
Proline	12.9	12.1	12.1	12.3	11.7
Alanine	11.4	11.6	11.3	11.3	11.2
Aspartic acid	21.2	23.2	22.4	22.8	21.8
Glutamic acid	36.6	39.1	37.0	38.6	37.9
Suppl. methionine	<0.1	0.13	1.18	1.72	-
Alimet	-	-	-	-	1.28

1. Analytical report from Degussa, 1996

* All amino acids were analysed by the oxidation of the protein hydrolysate except tryptophan which was by Alkaline hydrolysis with barium hydroxide, HPLC determination

** Supplemental amino acid (Methionine and lysine) were analysed by extraction with 0.1 N HCl

4.1.4 Livability

Only two birds died during experimental period i.e., one each on 6.6 g/kg and 9.0 g/kg TSAA.

It appears from these results that TSAA content of diet greater than 7.2 g/kg (methionine 4.2 g/kg) may not be beneficial in improving the performance of briolers from 8 to 21 d age.

Table 10: Analytical composition[1] experimental diets for methionine (Experiment I)

Diet	CP analysed g/kg	Supplemental DL-methionine Calculated g/kg	Supplemental DL-methionine Analysed g/kg
Reference	218	0.0	<0.1
Reference + 0.6 g/kg DLM	218	0.6	0.59
Reference + 1.2 g/kg DLM	218	1.2	1.44
Reference + 1.8 g/kg DLM	218	1.8	1.87
Reference + 2.4 g/kg DLM	218	2.4	2.73
Reference + 3.0 g/kg DLM	218	3.0	2.80
Reference + 3.6 g/kg DLM	218	3.6	4.21
Reference + 4.2 g/kg DLM	218	4.2	4.74

1 Analytical reports from Degussa, 1996

Table 11: Effects of dietary DL-methionine supplementaton on broiler performance (8-21 d) (Experiment 1)

Added methionine g/kg	Dietary methio-nine g/kg	Dietary TSAA level g/kg	Weight gain (g)	Feed intake (g/bird)	Feed : gain (g : g)
0	2.5	6.0	469^{a}	790^{a}	1.683^{a}
0.6	3.1	6.6	500^{a}	789^{a}	1.578^{b}
1.2	3.7	7.2	522^{a}	821^{a}	1.576^{b}
1.8	4.3	7.8	501^{a}	792^{a}	1.592^{b}
2.4	4.9	8.4	488^{a}	777^{a}	1.592^{b}
3.0	5.5	9.0	517^{a}	812^{a}	1.573^{b}
3.6	6.1	9.6	530^{a}	824^{a}	1.554^{b}
4.2	6.7	10.2	516^{a}	808^{a}	1.566^{b}
SEM			15.2	16.8	0.024

Means bearing the common superscripts in each column are not significantly different (P<0.05) from each other.

Analysis of variance

Source of variation	weight gain		Feed intake		Feed/gain	
	df	MSS	df	MSS	df	MSS
Between treatments	7	1975.44	7	1436.85	7	0.008*
Error	32	1161.79	32	1425.03	32	0.0029

df - degrees of freedom
MSS - Mean sum of squares

4.2 EXPERIMENT 2 - EVALUATION OF EFFICACY OF ALIMET^{R} AS METHIONINE SOURCE

Efficacy of Alimet$^{(R)}$ in relaton to DL-methionine was determined in male commercial broilers by including DL-methionine at 0, 1.3 g and Alimet$^{(R)}$ at 1.47 g, 2.0 g/kg diet from 9 to 28 d age. Assuming an efficacy of 88 per cent and 65 per cent, the supplemental Alimet$^{(R)}$ at 2.0 g/kg and 1.47 g/kg respectively was equal to the supplemental DL-methionine (1.3 g/kg). The methionine and TSAA of the diets is shown in Table 12 and supplemental amino acids in table 13. A good agreement exists between the supplemental and analysed values of DL-methionine and Alimet.

Performance of broiler chicks in terms of weight gain, feed intake and feed efficiency is presented in Table 14.

On reference diet the weight gains of chicks were significantly ($P<0.05$) lower than that on other diets. Alimet$^{(R)}$ at 1.47 g/kg improved the weight gains appreciably but not significantly over that on reference diet. Increasing the Alimet^{R} to 2 g/kg or supplementing the diet with DL-methionine at 1.3 g/kg significantly improved the weight gains compared to that on reference and Alimet^{R} 1.47 g/kg. There was no significant difference in the weight gains on Alimet$^{(R)}$ 2 g/kg and DL-methionine at 1.3 g/kg. Feed intake on Alimet^R at 1.47 g/kg was similar to that on reference diet. Supplemental Alimet^(R) at higher level (2 g/kg) and DL-methionine at 1.3 g/kg significantly increased feed intake over that of reference diet and Alimet^(R) at lower level (1.47 g/kg).

The feed/gain values on supplemental Alimet^(R) and DL-methionine diets were similar and significantly better than on reference diets.

The birds died during the experimental period were seven on reference diet, five on supplemental DL-methionine diet, three on supplemental Alimet^R at higher level (2 g/kg) and six on supplemental Alimet^R at lower level (1.47 g/kg). Much of this mortality was in the late experimental period due to heat.

Table 12: The methionine and TSAA content of diets used in experiment 2

Diet		Methionine g/kg	TSAA g/kg
Reference		3.0	6.2
Reference + DL-methionine 1.3 g/kg		4.3	7.5
Reference + Alimet^(R) 2 g/kg if efficacy	65%	4.3	7.5
	88%	4.7	7.9
Reference + Alimet^(R) 1.47 g/kg if efficacy	88%	4.3	7.5
	65%	3.9	7.1

Table 13: Analytical composition[1] of experimental diets for methionine and Alimet[R] (Experiment 2)

Diet	CP analysed g/kg	Supplemental DL-methionine analysed g/kg	Supplemental Alimet(R) analysed g/kg
Reference	228	-	-
Reference + 1.3 g/kg DL-methionine	225	1.18	-
Reference + 2 g/kg Alimet	225	-	1.72
Reference + 1.47 g/kg Alimet[R]	219	-	1.28

1 Analytical report from Degussa, 1996
CP - Crude protein

Table 14: Effects of dietary suplementation of DL-methionine and Alimet(R) on broiler performance (9-28 d) (Experiment 2)

Diet	Weight gain (g)	Feed intake (g/bird)	Feed/ gain	Livabi-lity %
Reference	637[a]	1220[a]	1.911[a]	93
Reference + DL-methionine, 1.3 g/kg	720[b]	1289[b]	1.787[b]	95
Reference + Alimet(R), 1.47 g/kg	664[a]	1226[a]	1.844[b]	94
Reference + Alimet[R] 2 g/kg	702[b]	1276[b]	1.818[b]	97
SEM	12.29	15.05	0.02	

* Alimet(R) at 1.47 g and 2 g/kg in diet at an assumed efficiency of 88% and 65% respectively, equals the activity of DL-methionine 1.3 g/kg.

Means bearing the common superscripts in each column are not significantly different (P<0.05) from each other.

Analysis of variance

Source of variation	Weight gain		Feed intake		Feed/gain	
	df	MSS	df	MSS	df	MSS
Between treatments	3	14021.72**	3	12335.72**	3	0.028**
Error	36	1510.71	36	2267.88	36	0.005

df - degrees of freedom

MSS - Mean sum of squares

DISCUSSION

CHAPTER V

DISCUSSION

The results on the requirement of total sulphur amino acids and efficacy of Alimet(R) in relation to DL-methionine are discussed in this chapter.

5.1 REQUIREMENT OF TSAA

Appreciable improvement in weight gains of broilers fed supplemental DL-methionine at 0.6 and 1.2 g/kg diet suggests that the requirement of TSAA may be 7.2 g/kg of the diet. At this level the methionine content of the diet was 3.7 g/kg representing 51.4 per cent TSAA.

The TSAA requirement values obtained in this study are similar to the values reported by Boomgaardt and Baker (1973). Nelson *et al.* (1960) reported a value 7.4 g/kg TSAA for broilers of 1 to 33 days age. The much lower values observed by many other research workers (Graber *et al.*, 1971; Willis and Baker, 1980; Robbins and Baker, 1980a,b, and Willis and Baker, 1981) could be due to lower genetic efficiency of the bird existing a decade back.

Ramasubba Reddy (1996) while reviewing the work done in India on the nutrient requirements of chicken suggested 5.4 g methionine and 8.8 g TSAA/kg diet at 2900 kcal ME/kg broiler of 1-21 d age. BIS (1994) recommended 5.0 g methionine and 9 g TSAA per kg diet at 2800 kcal

ME/kg for broiler during starting period. ICAR (1985) suggested a much lower requirement of 4.4 g methionine and 8.3 g TSAA per kg dose at 2900 kcal ME/kg. The NRC (1994) at 3200 kcal ME/kg recommended 5 g methionine and 9 g TSAA per kg diet.

5.2 EVALUATION OF EFFICACY OF ALIMET(R) AS METHIONINE SOURCE

Supplemental DL-methionine (1.3 g/kg) (dietary methionine 4.3 g, TSAA 7.5 g/kg) improved the weight gains, increased feed intake and improved feed/gain significantly over that on reference diet. The level of TSAA on this diet is 7.5 g/kg (methionine 4.3 g/kg, methionine as per cent of TSAA 57.3%) and this level is slightly higher than the requirement obtained in experiment 1.

Alimet was supplemented at 1.47 g and 2 g/kg. If it is assumed that the efficacy of Alimet(R) is only 65%, the level of supplemental Alimet(R) of 1.47 g/kg raises the dietary methionine to 3.96 g/kg and TSAA to 7.16 g/kg. This level is the requirement as observed in experiment I. At this level there was an appreciable improvement in weight gains and significant improvement in feed/gain over that on control. Further increase in the Aliment(R) level to 2 g/kg (dietary methionine 4.3 g and TSAA 7.5 g/kg) resulted in further increased weight gains, feed intake and feed/gain similar to that on DL-methionine supplemented diet. Probably, the requirement of TSAA is higher than 7.2 g/kg.

If the efficacy of Alimet(R) is assumed at 88%, the lowest level of Alimet(R) supplementation raises the TSAA similar to that on DL-methionine supplemented diet. On this basis, the performance would have been equal to that on DL-methionine supplemented diet. Infact the weight gains were significantly lower than on DL-methionine supplemented diet. Raising the supplemental level of Alimet(R) to 2 g/kg raises the TSAA content to 7.96 g/kg (methionine 4.76 g/kg). This level is higher than the requirement obtained in experiment I. At this level, improvement would not have been obtained over that on diet containing supplemental Alimet(R) at 1.47 g/kg, but the performance improved on increasing the Alimet(R) to 2 g/kg in terms of weight gain and feed intake. This clearly demonstrates that supplemental Alimet at 2 g/kg is equal to DL-methionine at 1.3 g/kg, indicating an efficacy of only 65% for Alimet(R) on weight basis.

The results obtained in this experiment are similar to an efficacy of 62 to 67 per cent obtained by van Weerden *et al.* (1982; 1983), Thomas et *al.* (1991), Huyghebeart (1993) and Rastagno and Barbosa (1995). On the other hand the results of waldroup *et al.* (1981), Elkin and Hester (1983) and Garlich (1985) did not find differences in methionine activity of Alimet(R) and DL-methionine on equimolar basis. These conflicting results may be due to differences in the diet used (van Weerden et al., 1982), existence of different polymeric forms of the MHA-FA (Boebel and Baker, 1982), poor sensitivity of the experiment and unsuitable evaluation of the data obtained (Huyghebeart, 1993).

In conclusion, the efficacy of Alimet(R) relative to DL-methionine may be about 65 per cent based on weight basis.

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SUMMARY

CHAPTER VI

SUMMARY

Studies were conducted to investigate the requirement of TSAA (experiment 1) and to determine the efficacy of Alimet R as methionine source (experiment 2) for commercial male broilers.

In experiment 1 (8-21 d), DL-methionine was included in appropriate quantities to a control diet containing 6 g/kg, TSAA (3 g methionine) to arrive at 6.6, 7.2, 7.8, 8.4, 9.0, 9.6 and 10.2 g/kg TSAA in diets. Each diet was fed to 5 groups of male broiler, 5 broilers per group from 8 to 21 days age. In experiment 2 (9-28d), DL- methionine and AlimetR assuming an activity at 65% and 88%, were included on weight basis in appropriate quantities to a control diet containing 6.2 g/kg TSAA (3.2 g methionine) to arrive at 7.5 g/kg TSAA. On each diet, 10 groups of 10 broiler males per group were placed from 9 to 28 days age. In both the experiments, the chicks were maintained in battery brooders feed and water were provided *adlib*.

In both the experiments performance was examined in terms of weight gains, feed intake feed efficiency and livability. From the results it was observed that:

1. Weight gains of broilers progressively increased up to 7.2 g/kg of TSAA (4.2 g methionine) in the

diet. Feed efficiency of supplemental DL-methionine diets was significantly $(P<0.05)$ better than that on control diet.

2. Supplemental Alimet at 1.47 g/kg resulted in higher weight gain than the reference diet. However the weight gain on this diet were significantly lower than on DL-methionine supplemented diet (1.3 g/kg) and Alimet 2 g/kg supplemented diet.

3. Supplemented DL-methionine at 1.3 g/kg and Alimet at 2 g/kg resulted in similar performance of broilers in terms of weight gain, feed intake and feed/gain.

4. Based on these observations, Alimet was assumed to have the efficacy of 65 per cent rather than 88 per cent.

In both the experiments, mortality lies within the normal range irrespective of dietary regimen.

It was inferred that the TSAA requirement of broilers is 7.2 g/kg in the diet (4.2 g methionine) and Alimet^R has an efficacy of 65% on weight basis.

LITERATURE CITED

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