IS 1374 (2007): Poultry Feeds [FAD 5: Livestock Feeds, Equipment and Systems]

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भारतीय मानक

कुक्कुट आहार — विशिष्टि

(पाँचवा पुनरीक्षण)

Indian Standard

POULTRY FEEDS — SPECIFICATION

(Fifth Revision)

ICS 65.120

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

June 2007

Price Group 11

FOREWORD

This Indian Standard (Fifth Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Livestock Feeds and Equipment Systems Sectional Committee had been approved by the Food and Agriculture Division Council.

This standard was first issued in 1959. Subsequently, in the light of research conducted in the country in the field of poultry nutrition and the changing raw material situation, this standard was revised in 1964, 1968, 1979 and in 1981. In the third revision, the scope of this standard was enlarged to include feeds meant for broilers and breeding chicken. Limits for characteristics to be declared by the manufacturers were also included.

The fourth revision of the standard was taken up to update the requirements on the basis of data available and to ensure that the recommended nutrient requirements were fulfilled.

The hen housed egg production has gone up from 260 eggs in 1965 to 320 eggs in 2004 and broiler weight has gone up from 1.5 kg in 8 weeks to 2.0 kg in 6 weeks. Thus, nutrient requirement of poultry need to be reviewed and keeping this in view, fifth revision of the standard has been taken up.

The composition of the Committee responsible for the formulation of this standard is given at Annex N.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

POULTRY FEEDS — SPECIFICATION

(Fifth Revision)

1 SCOPE

This standard prescribes requirement, sampling and methods of test for chicken (*Gallus domesticus*) feeds.

2 REFERENCES

The standards listed in Annex A contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent additions of the standards listed in Annex A.

3 TYPES

Chicken feeds shall be of the following fifteen types:

a) *Broiler Pre-starter Feed* (*BPSF*) — a ration to be fed to chicks, intended for meat production and to be used from 1 to 7 days.

b) *Broiler Starter Feed* (*BSF*) — a ration to be fed to growing chickens, intended for meat production, from 8 to 21 days.

c) *Broiler Finisher Feed* (*BFF*) — a ration to be fed to growing chickens, intended for meat production, from 22 days to finish.

d) *Chick Feed for Layer* (*CFL*) — a ration to be fed to chicks, intended for egg production, from 0 to 8 weeks.

e) *Grower Feed for Layer* (*GFL*) — a ration to be fed to growing chickens, intended for egg production, from 9 to 20 weeks or until laying commences.

f) *Layer Feed for Phase I* (*LFP-I*) — a ration to be fed to laying birds from 21 weeks to 45 weeks.

g) *Layer Feed for Phase II* (*LFP-II*) — a ration to be fed to laying birds from 46 weeks to 72 weeks. Phase II and I feed in layer cycle is necessary because there are changes in production, egg size, requirement of calcium, efficiency of digestion, age, etc.

h) *Breeder Chick Feed for Broiler* (*BCFB*) — a ration to be fed to chicks, intended for broiler breeding, from 0 to 4 weeks.

j) *Breeder Grower Feed for Broiler* (*BGFB*) — a ration to be fed to chickens, intended for broiler breeding, from 5 to 22 weeks.

k) *Breeder Layer Feed for Broiler* (*BLFB*) — a ration to be fed to laying birds, intended for broiler breeding, from week 23 onwards.

m) *Breeder Broiler Feed for Male* (*BBFM*) — a ration to be fed to male birds, intended for broiler breeding, from week 23 onwards.

n) *Chick Feed for Layer Breeder* (*CFLB*) — a ration to be fed to chicks, intended for layer breeding, from 0 to 4 weeks.

p) *Grower Feed for Layer Breeder* (*GFLB*) — a ration to be fed to chickens, intended for layer breeding, from 5 to 22 weeks.

q) *Breeder Layer Feed* (*BLF*) — a ration to be fed to laying birds, intended for layer breeding, from week 23 onwards.

r) *Breeder Layer Feed for Male* (*BLFM*) — a ration to be fed to male birds, intended for layer breeding, from week 23 onwards.

4 REQUIREMENTS

4.1 Description

Chicken feed shall be in the form of pellets, crumbs or mash. The feed shall be free from rancidity, musty odour, toxic ingredients, adulterants, moulds and insect infestation.

4.1.1 *Ingredients*

The ingredients listed in Annex B shall only be used for manufacturing chicken feeds.

4.1.2 The chicken feeds shall also conform to the requirements given in Tables 1 and 2.

4.1.3 Chicken feeds shall also contain the following vitamins, fatty acids, amino acids and minerals obtained either from the natural source or from any added source, in quantity not less than shown against each in Table 3.

4.1.4 The compound poultry feeds shall also conform to the maximum limits of harmful substances as given in Table 4.

4.2 Optional Requirements

4.2.1 It is recommended that chicken feeds may contain feed additives as given in Annex C.

4.2.2 The guidelines for quality of water used in feed management are given in Annex D.

4.2.3 The composition of commonly used poultry feed ingredients is given in Table 5.

4.2.4 The specification of poultry feed raw material is given in Tables 6 and 7.

4.2.5 The expected performance of chickens are given in Tables 8 and 9.

5 PACKING AND MARKING

5.1 Packing

The material shall be packed in clean, dry and sound, plain or polyethylene lined jute or laminated paper bags. The mouth of each bag shall be machine stitched.

5.2 Marking

Each bag shall be suitably marked so as to give the following information legibly:

a) Name of the material and brand name, if any;
b) Name and type of the poultry feed;
c) Name and address of the manufacturer;
d) Net mass when packed, in kg; and
e) Year and month of manufacture.

5.2.1 In addition to the information listed in **5.2**, each bag shall have a label or tag attached to it or contain a leaflet giving the following information:

a) Type of poultry feed;
b) Name and quantity of the antibiotic or coccidiostates added, if any;
c) Crude protein content;
d) Crude fibre content;
e) Aflatoxin B_1 content;
f) Metabolisable energy (calculated), in kcal/kg; and
g) The declaration that minerals, vitamins, fatty acids and amino acids are present in the material as per the specification.

NOTE — The manufacturer may declare the values of minerals, vitamins, fatty acids and amino acids from the record showing the quantities of these added to each batch.

5.3 BIS Certification Marking

The product may also be marked with the Standard Mark.

5.3.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standard Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in Annex E.

7 TESTS

7.1 Tests shall be carried out as prescribed in col 18 of Tables 1, 2 and 3.

7.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities, which affect the results of analysis.

Table 1 Requirements for Chicken Feeds

(Clauses 4.1.2, 7.1, E-4.2 *and* E-5)

Sl No.	Characteristic	Requirement for														Method of Test, Ref to Clause
		Broiler Feed			Layer Feed				Broiler Breeder Feed				Layer Breeder Feed				
		Pre-starter	Starter	Finisher	Chick	Grower	Layer Phase I	Layer Phase II	Chick	Grower	Layer	Male	Chick	Grower	*Rom*	Male	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
i)	Moisture, percent by mass, *Max*	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	**4** of IS 7874 (Part 1) or IS 14830
ii)	Crude protein (N × 6.25), percent by mass, *Min*	23.0	22.0	20.0	20.0	16.0	18.0	16.0	20.0	16.0	16.0	15.0	20.0	16.0	17.0	16.0	**5** of IS 7874 (Part 1) or IS 14825
iii)	Ether extract, percent by mass, *Min*	3.0	3.5	4.0	2.0	2.0	2.0	2.0	2.5	2.5	2.5	2.5	2.0	2.0	2.0	2.0	**7** of IS 7874 (Part 1)
iv)	Crude fibre, percent by mass, *Max*	5.0	5.0	5.0	7.0	9.0	9.0	10.0	7.0	9.0	9.0	9.0	7.0	9.0	9.0	9.0	**8** of IS 7874 (Part 1) or IS 10226 (Part 1)
v)	Acid insoluble ash, percent by mass, *Max*	2.5	2.5	2.5	4.0	4.0	4.0	4.5	4.0	4.0	4.0	4.0	2.5	2.5	2.5	2.5	**10** of IS 7874 (Part 1) or IS 14826
vi)	Salt (as NaCl), percent by mass, *Max*	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	**4** of IS 7874 (Part 2)

NOTES

1 The values specified for characteristics at Sl No. (ii) to (vi) are on dry matter basis.

2 Earlier the broiler cycle was for eight weeks. It has now reduced to 6 weeks. The split is therefore 0 to 3 weeks starter and 4 to 6 weeks finisher. The starter period is further split into pre-starter 0 to 7 days and starter 8 to 21 days. This optimizes the performance. Therefore it is advised that Prestarter Feed to be used from 1 to 7 days. Starter feed from 8 to 21 days and Finisher Feed from 22 days to finish.

3 An expected broiler performance as on current status is given in Tables 8 and 9. It must be noted that the performance parameters may change with the input of high genetic material in future. These values are applicable as on current basis and may only be viewed as guidelines.

4 It has been observed that with the increasing usage of essential amino acids such as lysine and methionine the need for high protein has come down significantly. This has resulted into lowering of protein content in the broiler feed lines.

5 Studies are being conducted on the role of threonine and tryptophan. If they are available commercially to improve performance, the protein values can be further changed in future.

6 The energy values have been increased as compared to existing Indian Standards, because of current feed efficiency of 1.8 as compared to previous 2.2. The feed being manufactured now-a-days is denser with high energy.

Table 1 (*Concluded*)

7 Ether Extract means all ether soluble materials, which include oil, alcohol, cholesterol, pigments etc. Since the method for estimation exclusively for oil is not available, ether extract connotation is being used

8 In earlier feeds use of methionine and choline was limited. This incorporation has gone up and since both help as lypolytic factors, it is felt that the role of biotin is limited, hence the biotin values have been reduced compared to existing Indian Standards.

9 Chick stage is 0 to 8 weeks. Grower stage is 9 to 20 weeks. Phase I is from 21 weeks to 45 weeks and Phase II is from 46 weeks to 72 weeks of age of the bird. It is therefore advised to use the respective feeds accordingly

10 Phase I and Phase II in layer cycle is necessary because there are changes in production, egg size, requirement of calcium, efficiency of digestion, age, etc.

11 Top dressing of extra calcium source in the form of shell grit/limestone at about 4-5 g per bird per day is advised in case of laying stage both in Phase I and Phase II feeds.

12 The expected performance of layers has been furnished in Table 9, which may be used as guideline, depending upon the present genetic potential of the bird.

13 It is advised to use chick feed from 0-4 weeks, grower feed from 5-22 weeks and layer breeder feed and male breeder feed from week 23 onwards.

14 Top dressing of extra calcium source in the form of shell grit/limestone at about 4-5 g per bird per day is advised in case of laying stage both in Phase I and Phase II feeds.

Table 2 Requirements for Chicken Feeds

(*Clauses* 4.1.2 *and* 7.1)

Sl No.	Characteristic	Requirement for															Method of Test, Ref to
		Broiler Feed			Layer Feed				Broiler Breeder Feed				Layer Breeder Feed				
		Pre-starter	Starter	Finisher	Chick	Grower	Layer Phase I	Layer Phase II	Chick	Grower	Layer	Male	Chick	Grower	*Layer*	Male	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
i)	Calcium (as Ca), percent by mass, *Min*	1.0	1.0	1.0	1.0	1.0	3.0	3.5	1.0	1.0	3.5	1.0	1.0	1.0	3.5	1.0	IS 13433 (Parts 1 and 2)
ii)	Total phosphorus, percent by mass, *Min*	0.7	0.7	0.7	0.7	0.65	0.65	0.65	0.70	0.70	0.70	0.70	0.65	0.60	0.60	0.60	Clause 6 of IS 7874 (Part 2) or IS 14828
iii)	Available phosphorus, percent by mass, *Min*	0.45	0.45	0.45	0.45	0.40	0.40	0.40	0.45	0.45	0.40	0.40	0.45	0.40	0.40	0.40	Annex F
iv)	Lysine, percent by mass, *Min*	1.3	1.2	1.0	1.0	0.7	0.7	0.65	1.0	0.8	0.85	0.80	0.95	0.70	0.70	0.80	Annex G
v)	Methionine, percent by mass, *Min*	0.5	0.5	0.45	0.40	0.35	0.35	0.30	0.45	0.40	0.45	0.40	0.40	0.40	0.40	0.40	Annex H
vi)	Methionine + Cystine, percent by mass, *Min*	0.9	0.9	0.85	0.70	0.60	0.60	0.55	0.70	0.70	0.70	0.70	0.70	0.60	0.60	0.60	IS 1374
vii)	Metabolizable energy (Kcal/kg), *Min*	3 000	3 100	3 200	2 800	2 500	2 600	2 400	2 800	2 750	2 800	2 750	2 800	2 600	2 600	2 600	Annex J
viii)	Aflatoxin B_1 (ppb), *Max*	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	Annex K or IS 13427

NOTES

1 The values specified for characteristics at Sl No. (ii) to (vi) are on dry matter basis.

2 IS 14828 shall be the referee's method, in case of dispute.

3 In case of dispute, HPLC method shall be the referee's method.

4 Earlier the broiler cycle was for eight weeks. It has now reduced to 6 weeks. The split is therefore 0 to 3 weeks starter and 4 to 6 weeks finisher. The starter period is further split into pre-starter 0 to 7 days and starter 8 to 21 days. This optimizes the performance. Therefore it is advised that Prestarter Feed to be used from 1 to 7 days, Starter feed from 8 to 21 days and Finisher Feed from 22 days to finish.

5 An expected broiler performance as on current status is given in Tables 8 and 9. It must be noted that the performance parameters may change with the input of high genetic material in future. These values are applicable as on current basis and may only be viewed as guidelines.

6 It has been observed that with the increasing usage of essential amino acids such as lysine and methionine the need for high protein has come down significantly. This has resulted into lowering of protein content in the broiler feed lines.

Table 2 (*Concluded*)

7 Studies are being conducted on the role of threonine and tryptophan. If they are available commercially to improve performance, the protein values can be further changed in future.

8 The energy values have been increased as compared to existing Indian Standards, because of current feed efficiency of 1.8 as compared to previous 2.2. The feed being manufactured now-a-days is denser with high energy.

9 Ether Extract means all ether soluble materials, which include oil, alcohol, cholesterol, pigments etc Since the method for estimation exclusively for oil is not available, ether extract connotation is being used.

10 In earlier feeds use of methionine and choline was limited. This incorporation has gone up and since both help as lypolytic factors, it is felt that the role of biotin is limited, hence the biotin values have been reduced compared to existing Indian Standards.

11 Chick stage is 0 to 8 weeks. Grower stage is 9 to 20 weeks. Phase I is from 21 weeks to 45 weeks and Phase II is from 46 weeks to 72 weeks of age of the bird. It is therefore advised to use the respective feeds accordingly.

12 Phase I and Phase II in Layer cycle is necessary because there are changes in production, egg size, requirement of calcium, efficiency of digestion, age etc.

13 Top dressing of extra calcium source in the form of shell grit/limestone at about 4-5 g per bird per day is advised in case of laying stage both in Phase I and Phase II feeds.

14 The expected performance of layers has been furnished in Table 9, which may be used as guideline, depending upon the present genetic potential of the bird.

15 It is advised to use Chick Feed from 0-4 weeks, Grower Feed from 5-22 weeks and Layer Breeder Feed and Male Breeder Feed from week 23 onwards.

16 Top dressing of extra calcium source in the form of shell grit/limestone at about 4-5 g per bird per day is advised in case of laying stage both in Phase I and Phase II feeds.

Table 3 Requirements for Minerals, Vitamins, Amino Acids and Fatty Acids per kg of Chicken Feeds

(*Clauses* 4.1.3 *and* 7.1)

Sl No.	Characteristic	Requirement for															Method of Test, Ref to
		Broiler Feed			Layer Feed				Broiler Breeder Feed				Layer Breeder Feed				
		Pre-starter	Starter	Finisher	Chick	Grower	Layer Phase I	Layer Phase II	Chick	Grower	Layer	Male	Chick	Grower	*Layer*	Male	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
i)	Manganese, mg/kg, *Min*	100.0	100.0	100.0	70.0	60.0	60.0	60.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	IS 15121
ii)	Iodine, mg/kg, *Min*	1.2	1.2	1.2	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	IS 15121
iii)	Iron, mg/kg, *Min*	80.0	80.0	80.0	70.0	60.0	60.0	60.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	IS 15121
iv)	Zinc, mg/kg, *Min*	80.0	80.0	80.0	60.0	60.0	60.0	60.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	IS 15121
v)	Copper, mg/kg, *Min*	12.0	12.0	12.0	12.0	9.0	9.0	9.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	IS 15121
vi)	Selenium, mg/kg, *Min*	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.20	0.15	0.15	0.15	0.20	0.15	IS 15121
vii)	Vitamin A, IU/kg, *Min*	11 000	11 000	10 000	9 000	8 000	8 000	8 000	12 000	12 000	15 000	12 000	12 000	12 000	15 000	12 000	IS 15120
viii)	Vitamin D_3, IU/kg, *Min*	3 000	3 000	3 000	1 800	1 600	1 600	1 600	2 500	2 500	3 000	2 500	2 500	2 500	3 000	2 500	Annex L
ix)	Vitamin B_1, mg/kg, *Min*	2.5	2.5	2.5	2.0	1.5	1.0	1.0	2.0	2.0	3.0	2.0	2.0	2.0	3.0	2.0	IS 5398
x)	Vitamin B_2, mg/kg, *Min*	6.0	6.0	6.0	6.0	5.0	5.0	5.0	5.0	5.0	6.0	5.0	5.0	5.0	6.0	5.0	IS 5399
xi)	Pantothenic acid, mg/kg, *Min*	15.0	15.0	15.0	10.0	9.0	7.0	7.0	15.0	15.0	25.0	15.0	15.0	15.0	25.0	15.0	IS 9840
xii)	Niacin, mg/kg, *Min*	40.0	40.0	40.0	40.0	20.0	20.0	20.0	40.0	40.0	50.0	40.0	40.0	40.0	50.0	40.0	IS 5400
xiii)	Biotin, mg/kg, *Min*	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	IS 9820
xiv)	Vitamin B_{12}, mg/kg, *Min*	0.015	0.015	0.015	0.010	0.008	0.008	0.008	0.025	0.025	0.030	0.025	0.025	0.025	0.030	0.025	IS 7529
xv)	Folic acid, mg/kg, *Min*	1.0	1.0	1.0	1.0	0.50	0.50	0.50	3.0	3.0	4.0	3.0	3.0	3.0	4.0	3.0	IS 7234
xvi)	Choline, mg/kg, *Min*	500.0	500.0	500.0	500.0	200.0	400.0	400.0	850.0	850.0	700.0	500.0	850.0	850.0	700.0	500.0	IS 7874
xvii)	Vitamin E, mg/kg, *Min*	30.0	30.0	30.0	15.0	10.0	10.0	10.0	20.0	20.0	50.0	20.0	20.0	20.0	50.0	20.0	Annex M
xviii)	Vitamin K, mg/kg, *Min*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.0	2.0	3.0	2.0	2.0	2.0	3.0	2.0	IS 7874
xix)	Vitamin B_6, mg/kg, *Min*	5.0	5.0	5.0	3.0	3.0	3.0	3.0	5.0	5.0	6.0	5.0	5.0	5.0	6.0	5.0	IS 7530
xx)	Linoleic acid, percent by mass, *Min*	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	IS 7874

NOTES

1 The values specified for characteristics at Sl No. (i) to (xx) are on dry matter basis.

2 Earlier the broiler cycle was for eight weeks. It has now reduced to 6 weeks. The split is therefore 0 to 3 weeks starter and 4 to 6 weeks finisher. The starter period is further split into pre-starter 0 to 7 days and starter 8 to 21 days. This optimizes the performance. Therefore it is advised that Prestarter Feed to be used from 1 to 7 days. Starter feed from 8 to 21 days and Finisher Feed from 22 days to finish.

Table 3 (*Concluded*)

3 An expected broiler performance as on current status is given in Tables 8 and 9. It must be noted that the performance parameters may change with the input of high genetic material in future. These values are applicable as on current basis and may only be viewed as guidelines.

4 It has been observed that with the increasing usage of essential amino acids such as lysine and methionine the need for high protein has come down significantly. This has resulted into lowering of protein content in the broiler feed lines.

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Table 4 Maximum Prescribed Limit for Harmful Substances and Test Methods in Compound Poultry Feeds

(*Clauses* 4.1.4, E-2.3 *and* E-3.1)

Sl No.	Substance	Feed	Max Content in mg/kg Referred into a Moisture Content of 11 Percent	Test Methods
(1)	(2)	(3)	(4)	(5)
i)	Arsenic	Complete feed for poultry	2	A.O.A.C.
ii)	Fluorine	Complete feed for poultry	30	A.O.A.C.
iii)	Lead	Complete feed for poultry	5	A.O.A.C.
iv)	Mercury	Complete feed for poultry	Nil	A.O.A.C.
v)	Nitrite (Na nitrite)	Complete feed for poultry	15	A.O.A.C.
vi)	Aflatoxin B_1	Complete feed for poultry	0.02	A.O.A.C.
vii)	Castor	Complete feed for poultry	10	A.O.A.C.
viii)	Free gossypol	Complete feed for poultry	20	A.O.A.C.
ix)	Hydrocyanic acid	Complete feed for poultry	10	A.O.A.C.
x)	BHC	Complete feed for poultry	20 ppb	A.O.A.C.
xi)	DDT	Complete feed for poultry	5 ppb	A.O.A.C.
xii)	Endosulphan	Complete feed for poultry	10 ppb	A.O.A.C.
xiii)	Aldrin	Complete feed for poultry	1 ppb	A.O.A.C.

NOTE — Based on the adequate information available on all other mycotoxins such as Ochratoxins, Citrinine, T2 Toxin, Fusarium, Zearalenone, etc, their limits in the existing Indian Standards will be considered later.

Table 5 Composition of Commonly Used Feed Ingredients — Poultry

(*Clause* 4.2.3)

Sl No.	Ingredient	ME	Proximate Specification (Percent)					Amino Acids Specification (Percent)										
			Crude	Crude	Crude	Total	Sand	Lysine		Methionine		Methionine + Cystine		Tryptophan		Threonine		Linoleic Acid
		kcal/kg	Protein	C. Fat	Fibre	Ash	Silica	Total	Dig[1)]	Total	Digest	Total	Digest	Total	Digest	Total	Digest	
	Energy Ingredient																	
i)	Maize	3 300	9	4	2	1.5	1	0.27	0.22	0.17	0.16	0.37	0.34	0.08	0.06	0.31	0.25	1
ii)	*Jowar*	3 000	10	3	4	3	1	0.22	0.16	0.17	0.15	0.38	0.28	0.1	0.07	0.31	0.24	1.1
iii)	*Bajara*	2 640	12.7	4.9	2.2	2	1	0.42	NA	0.24	NA	0.38	NA	0.18	NA	0.44	NA	NA
iv)	Rice (broken)	2 600	7.9	1.7	1.4	4	2	0.26	0.21	0.2	0.17	0.37	0.31	0.09	NA	0.26	0.21	0.6
v)	Wheat	3 100	14	2.6	2.5	2	1	0.38	0.31	0.21	0.18	0.5	0.44	0.15	NA	0.38	0.32	2
vi)	Barley Grain	2 640	11.5	1.9	5	2.5	1	0.44	0.34	0.18	0.15	0.42	0.34	0.12	NA	0.53	0.45	1
vii)	Rice Polish	2 700	12.7	14	5	8	2.5	0.54	0.4	0.24	0.18	0.5	0.36	0.14	NA	0.45	0.31	4.4
viii)	*Ragi*	2 950	12.6	1.85	2.8	1.45	0.5	0.36	0.24	0.15	0.2	0.36	0.26	—	—	0.32	0.3	—
ix)	Tapioca Flour	3 300	2	0.7	12.9	8.5	—	0.06	—	0.006	—	0.01	—	—	—	—	—	—
x)	Vegetable Fat	8 800	—	99.4	—	—	—	—	—	—	—	—	—	—	—	—	—	31.0
	Protein Source																	
xi)	Soy Extract	2 500	48	1	3.5	5	2	2.98	2.68	0.69	0.63	1.41	1.24	0.61	0.57	1.89	1.68	0.4
xii)	Soy Meal	2 250	44	0.8	6.5	6	2.5	2.75	2.48	0.64	0.59	1.31	1.15	0.57	NA	1.76	1.57	0.4
xiii)	Groundnut Extract	2 690	48	1.5	6.8	7.2	2.5	1.77	NA	0.42	NA	1.15	NA	0.5	NA	1.16	—	0.19
xiv)	Groundnut Meal	2 400	44	1	10	8	2.5	1.39	1.07	0.42	0.37	1.1	0.9	0.41	NA	1.12	0.94	0.19
xv)	Rapeseed Extract	1 900	36	1.7	11.5	7	2	2.02	1.61	0.73	0.65	1.64	1.33	0.47	0	1.58	1.26	0
xvi)	Sunflower Extract	1 540	28	1	24	7.7	2	1.06	0.86	0.67	0.62	1.15	0.99	0.34	0	1.05	0.89	0.5
xvii)	Sesame Extract	2 200	44	0.5	6.1	11.5	2	1.01	0.83	1.16	0.98	1.97	1.6	0.54	0	1.44	1.05	1.9
xviii)	Fullfat Soy	3 300	38	18	5	4.6	2	2.37	1.9	0.51	0.4	1.16	0.83	0.69	0.45	1.57	1.21	7.7
xix)	Maize Gluten 42	3 150	42	2	4	3	1.5	0.8	0.63	1.0	0.85	1.6	1.33	0.2	0.17	1.1	1.06	—
xx)	Maize Gluten 60	3 650	60	2	2.5	1.3	0.5	1.1	0.85	1.5	1.3	2.3	2.0	0.3	0.25	1.6	1.32	—
xxi)	Fishmeal	2 180	45	7	1	22	5	2.5	2.8	0.84	0.7	1.15	0.86	0.7	—	1.4	1.2	—
xxii)	Meat & Bonemeal	1 848	45	8.6	2.1	38	—	2.48	2.12	0.65	0.59	1.16	0.94	0.29	0.23	1.6	1.36	0.3

Table 5 (*Concluded*)

Sl No.	Ingredient	ME	Proximate Specification (Percent)					Amino Acids Specification (Percent)										
			Crude	Crude	Crude	Total	Sand	Lysine		Methionine		Methionine + Cystine		Tryptophan		Threonine		Linoleic Acid
		kcal/kg	Protein	C. Fat	Fibre	Ash	Silica	Total	Dig[1)]	Total	Digest	Total	Digest	Total	Digest	Total	Digest	
	Others																	
xxiii)	Ricebran Doc	1 800	16	0.5	14	12.5	5	0.66	0.48	0.31	0.23	0.64	0.44	0.17	—	0.54	0.37	—
xxiv)	Wheat Bran	1 400	14.5	3	11	7	2	0.63	2.12	0.23	0.19	0.56	0.44	0.28	0.21	0.52	0.38	1.7
xxv)	Salseed Extract	2 500	9	1	3	—	1.5	0.5	0.38	0.32	0.24	0.52	0.4	0.1	0.07	0.3	0.23	0.3
xxvi)	Molasses	2 000	3	—	—	9.5	—	—	—	—	—	—	—	—	—	—	—	—

NOTE — Analysis not available.

[1)] Digestible.

Table 6 Composition of Commonly Used Feed Ingredients: Natural Vitamins

(*Clause* 4.2.4)

Raw Material	Riboflavin	Niacin	Pantothenic Acid	Thiamin	Choline	Biotin	Folic Acid	Pyridoxine	Carotine	Xanthophil	Alfa Tocopherol	Vit. A IU/kg
Energy Ingredient												
Maize	1.10	19.80	3.96	3.3	506	0.07	0.29	4.62	1.98	15.4	11.00	3 300
Jowar	1.20	42.70	11.00	3.9	678	0.18	0.02	NA	NA	NA	NA	0
Bajara	1.60	52.60	7.40	6.6	789	NA	NA	NA	NA	NA	NA	NA
Rice (broken)	0.88	28.60	7.92	2.64	792	0.07	0.18	4.40	0	0	8.80	0
Wheat	1.10	48.40	9.02	4.4	1 078	0.11	0.42	2.20	0	0	8.80	0
Barley Grain	1.54	52.80	7.92	4.84	1 100	0.04	0.66	2.86	0	0	7.04	0
Rice Polish	1.76	407.00	46.20	19.8	1 232	0.62	NA	13.20	NA	NA	90.20	NA
Ragi	1.50	15.60	9.20	4.4	0	0.06	0.60	0.00	0	0	0	0
Tapioca Flour	0	0	0	0	0	0	0	0	0	0	0	0
Vegetable Fat	0	0	0	0	0	0	0	0	0	0	0	0
Protein Source												
Soy Extract	2.86	26.40	15.40	4.62	2 640	0.33	0.66	5.94	—	—	1.96	—
Groundnut Extract	7.70	165.00	46.20	5.94	1 892	0.37	0.40	8.8	—	—	2.86	300
Rapeseed Extract	3.52	158.40	9.46	5.06	6 699	0.88	2.20	7.04	—	—	—	—
Sunflower Extract	2.2	198.00	28.60	2.86	2 860	0.66	1.32	11.00	—	—	11	7
Sesame Extract	3.30	28.60	5.50	2.64	1 496	0.33	0.00	12.54	—	—	—	—
Fullfat Soy	2.64	22.00	11.00	4.62	2 860	0.29	4.18	10.78	—	—	9.24	0
Maize Gluten 42	1.50	54.50	9.60	0.22	330	0.15	0.22	3.6	16.2	—	—	25
Maize Gluten 60	2.20	81.00	2.90	0.28	2 200	0.22	0.23	2.8	44		—	60
Fishmeal	6.60	66.00	8.80	1.32	3 060	0.09	0.20	0	—	—	—	—
Meat and Bonemeal (50%)	1.98	35.20	4.62	0.44	1 320	0.04	0.22	—	—	—	—	—
Others												
Ricebran Doc	0.88	28.60	7.92	2.64	792	0.07	0.18	4.4	—	—	8.8	—
Wheat Bran	3.08	184.80	28.60	6.60	1 870	0.22	1.19	6.82	0	0	13.2	0
Salseed Extract	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Molasses	2.86	33	37.4	0.88	748	0.66	0.09	4.18	NA	NA	7.04	NA

Table 7 Poultry Feed Raw Material Specification: Macro and Micro Minerals

(*Clause* 4.2.4)

Raw Material	Macro Minerals (Percent)							Micro Minerals (mg/kg)					
	Calcium	Total Phosph.	Avail. Phosph.	Sodium	Chloride	Potassium	Magnesium	Copper	Iron	Manganese	Zinc	Sulphur	Selenium
Energy Ingredient	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Maize	0.01	0.25	0.08	0.01	0.04	0.42	0.11	2	40	7	10	1 100	0.04
Jowar	0.04	0.40	0.13	0.01	0.1	0.4	0.13	10	40	10	10	900	0
Bajara	0.13	0.72	0.36	0.04	0	0.43	0.16	21.6	40	29	13.9	1 300	0
Rice (broken)	0.11	0.48	0.24	0.04	0	0.34	0.14	3.3	40	17.6	1.8	50	0
Wheat	0.05	0.28	0.14	0.02	0.05	0.35	0.15	5	50	38	30	1 400	0.065
Barley Grain	0.08	0.42	0.21	0.03	0.18	0.56	0.12	10	70	16	15	1 500	0.35
Rice Polish	0.27	1.37	0.14	0.06	0.07	1.7	0.95	13	190	130	30	1 800	0
Ragi	0.08	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tapioca Flour	0.58	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vegetable Fat	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Protein Source													
Soy Extract	0.40	0.60	0.14	0.02	0.05	2.00	0.27	21	100	27	40	4 300	0.01
Groundnut Extract	0.10	0.60	0.20	0.07	0.00	1.15	0.33	0	0	26	21.7	2 800	0
Rapeseed Extract	0.60	1.10	0.25	0.03	0.10	1.20	0.64	6	160	53	71	2 500	0.98
Sunflower Extract	0.35	0.90	0.30	0.01	0.05	1.00	0.62	35	140	34	100	3 800	1.5
Sesame Extract	2.00	1.30	0.24	0.04	0.00	1.39	0.00	0	0	48	0	0	0
Fullfat Soy	0.25	0.59	0.20	0.04	0.08	1.70	0.21	15	75	30	40	3 000	0.1
Maize Gluten 42	0.16	0.40	—	0.10	—	0.03	0.06	28.2	400	7.3	—	600	1
Maize Gluten 60	0.02	0.70	—	0.03	—	0.45	0.15	22	167	4.4	41	600	1
Fishmeal	7.16	1.67	1.67	0.19	0.60			21.7	320	38.9			0
Meat and Bonemeal (45%)	10.00	5.10	5.10	0.60	0.00	1.30	1.00	0	500	10.1	0	2 600	0.25
Others													
Ricebran Doc	0.37	1.80		0.06	0.07	1.70	0.95	13	190	130	30	1 800	0
Wheat Bran	0.08	1.15	0.40	0.02	0.06	1.23	0.50	14	170	100	9.5	2 200	1
Salseed Extract	0.24	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Molasses	0.80	0.08	0.04	0.16	2.31	2.8	0.35	50	180	40	20	3 500	0
Minerals													
Limestone Powder	38.00	0.00	0.00	0.05	0.03	0.10	2.10	0	3.5	0	0	0	0
Dicalcium Phosphate	23.00	18.00	18.00	0.06	0.01	0.10	0.60	70	500	120	380	11 100	0
Sodium chloride	0.30	0.00	0.00	39.00	60.00	0.00	0.01	0	50	0	0	2 000	0
Soda Bi Carb		0.00	0.00	27.00	0.00	0.00	0.00	0	0	0	0	0	0
Sheli Grit	38.00	0.10	0.10	0.20	0.01	0.10	0.30	0	2.9	100	0	0	0
Bone Meal	29.80	12.50	12.50	0.04	0.00	0.20	0.30	0	5	0	100	24 000	0

Table 8 Expected Performance of Broilers (Typical Body Weights, Feed Requirements and Energy Consumption of Broilers)

(*Clause* 4.2.5, and *Tables* 1, 2 *and* 3)

Sl No.	Age (Weeks)	Body Weight			Weekly Feed Consumption			Cummulative Feed Consumption			Weekly Energy Consumption Cal ME/Bird			Cummulative Energy Consumption ME/Bird		
		Male	Female	Avg.	Male	Female	Avg.	Male	Female	Avg.	Male	Female	Avg.	Male	Female	Avg.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
i)	1	152	144	148	134	131	132.5	135	131	133	395	386	391	395	386	391
ii)	2	376	344	360	290	273	281.5	425	404	414.5	856	805	830	1 251	1 191	1 221
iii)	3	686	617	651.5	487	444	465.5	912	848	880	1 437	1 310	1 373	2 686	2 501	2 594
iv)	4	1 085	965	1 025	704	642	673	1 616	1 490	1 553	2 077	1 894	1 985	4 765	4 341	4 579
v)	5	1 576	1 344	1 046	960	738	849	2 576	2 228	2 402	2 925	2 251	2 590	7 693	6 592	7 169
vi)	6	2 088	1 741	1 915	1 141	1 001	1 071	3 717	3 229	3 473	3 480	3 503	3 267	11 173	9 645	10 436
vii)	7	2 590	2 134	2 362	1 281	1 081	1 181	4 998	4 310	4 654	3 907	3 297	3 602	15 080	12 972	14 038

Table 9 Expected Performance of Commercial Layer Flocks

(*Clause* 4.2.5, and *Tables* 1, 2 *and* 3)

Sl No.	Age in Weeks	Production (Percent)	Egg/HH (Week)	Egg (HH)	Cummulative Daily
(1)	(2)	(3)	(4)	(5)	(6)
i)	19	5	0.35	0.35	75
ii)	20	15	1.05	1.40	62
iii)	21	38	2.66	4.06	90
iv)	22	64	4.48	8.54	93
v)	23	83	5.60	14.34	96
vi)	24	80	6.22	20.55	102
vii)	25	92	5.43	26.99	104
viii)	26	94	6.57	33.56	106
ix)	27	94	6.56	40.12	108
x)	28	95	6.63	46.75	108
xi)	29	96	6.69	53.44	109
xii)	30	97	6.76	60.20	111
xiii)	31	97	6.76	66.96	111
xiv)	32	97	6.76	73.20	115
xv)	33	96	6.68	80.40	115
xvi)	34	96	6.67	87.07	115
xvii)	35	96	6.67	93.73	114
xviii)	36	96	6.66	100.39	114
xix)	37	95	6.58	106.98	114
xx)	38	95	6.58	113.55	113
xxi)	39	95	6.58	120.13	113
xxii)	40	95	6.58	126.69	113
xxiii)	41	94	6.59	133.18	113
xxiv)	42	94	6.68	139.66	113
xxv)	43	94	6.47	146.13	113

Table 9 (*Concluded*)

Sl No.	Age in Weeks	Production (Percent)	Egg/HH (Week)	Egg (HH)	Cummulative Daily
(1)	(2)	(3)	(4)	(5)	(6)
xxvi)	44	93	6.40	152.53	113
xxvii)	45	93	6.39	158.92	113
xxviii)	46	93	6.39	165.31	113
xxix)	47	93	6.38	171.69	113
xxx)	48	93	6.37	178.06	113
xxxi)	49	92	6.30	184.36	113
xxxii)	50	92	6.29	190.65	112
xxxiii)	51	91	6.22	196.87	112
xxxiv)	52	90	6.14	203.01	112
xxxv)	53	89	6.07	209.06	112
xxxvi)	54	89	6.07	215.15	112
xxxvii)	55	89	6.06	221.21	112
xxxviii)	56	89	6.06	227.27	112
xxxix)	57	89	6.06	233.33	112
xl)	58	86	5.98	239.31	112
xli)	59	89	5.98	245.29	112
xlii)	60	60	5.97	251.26	112
xliii)	61	68	5.97	257.22	110
xliv)	62	87	5.90	263.12	110
xlv)	63	87	5.89	269.02	110
xlvi)	64	86	5.82	274.84	110
xlvii)	65	86	5.81	280.65	110
xlviii)	66	86	5.81	285.46	110
xlix)	67	85	5.74	292.20	110
l)	68	84	5.66	297.86	110
li)	69	84	5.66	303.52	110
lii)	70	83	5.59	309.11	110
liii)	71	82	5.52	314.63	110
liv)	72	81	5.44	320.07	110

NOTE — 'Egg Yield 320 Eggs', Feed Consumption 41.25 kg during laying period.

ANNEX A

(*Clause* 2)

LIST OF REFERRED INDIAN STANDARDS

IS No.	*Title*
920 : 1972	Common salt for animal consumption including cattlelicks mineralized (*first revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
1162 : 1958	Specification for cane molasses
1712 : 1982	Cottonseed oilcake as livestock feed ingredient (*second revision*)
1713 : 1986	Specification for decorticated groundnut oilcake as livestock feed ingredient (*second revision*)
1932 : 1986	Specification for mustard and rapeseed oilcake as livestock feed ingredient (*second revision*)
1934 : 1982	Specification for sesamum oilcake as livestock feed ingredient (*second revision*)
2151 : 1985	Specification for maize germ oilcake as livestock feed ingredient (*first revision*)
2152 : 1972	Specification for maize gluten feed (*first revision*)
2154 : 1986	Specification for coconut oilcake as livestock feed ingredient (*second revision*)
2503 : 1986	Specification for decorticated safflower (*Kardi*) oilcake as livestock feed ingredient (*first revision*)
3441 : 1982	Specification for solvent extracted groundnut oilcake (meal) as livestock feed ingredient (*first revision*)
3592 : 1985	Specification for solvent extracted decorticated cottonseed oilcake (meal) as livestock feed ingredient (*second revision*)
3593 : 1979	Specification for solvent extracted rice bran as livestock feed (*second revision*)
3648 : 1975	Specification for rice bran as livestock feed
4193 : 1986	Specification for guar meal as livestock feed ingredient (*first revision*)
4307 : 1983	Specification for fishmeal as livestock feed ingredient (*second revision*)
4905 : 1968	Methods for random sampling
5065 : 1986	Specification for meat meal as livestock feed ingredient (*first revision*)
5398 : 1969	Methods for estimation of thiamine (Vitamin B-1) in foodstuffs
5399 : 1969	Methods for estimation of riboflavin (Vitamin B-2) in foodstuffs
5400 : 1969	Methods for estimation of nicotinic acid (Niacin) in foodstuffs
5470 : 2002	Specification for dicalcium phosphate, animal feed grade (*first revision*)
6242 : 1985	Specification for solvent extracted undecorticated safflower oilcake (meal) as livestock feed ingredient (*first revision*)
7059 : 1973	Specification for solvent extracted salseed meal as livestock feed ingredient
7060 : 1973	Specification for blood meal as livestock feed ingredient
7234 : 1974	Method for estimation of folic acid in foodstuffs
7529 : 1975	Method for estimation of Vitamin B-12 in foodstuffs
7530 : 1975	Method for estimation of pyridoxine (Vitamin B-6) in foodstuffs
7874	Methods of test for animal feeds and feeding stuffs
(Part 1) : 1975	General methods
(Part 2) : 1975	Mineral and trace elements
9820 : 1981	Method for estimation of biotin in foodstuffs
9840 : 1981	Method for estimation of pantothenic acid in foodstuffs
10147 : 1982	Specification for undecorticated sunflower oilcake as livestock feed ingredient
10165 : 1982	Specification for decorticated sunflower oilcake as livestock feed ingredient
10226 (Part 1) : 1982	Method for determination of crude fibre content: Part 1 General method

IS No.	*Title*
10657 : 1983	Specification for solvent extracted soyabean oilcake (meal) as livestock feed ingredient
12829 : 1989	Mango seed kernel (solvent extracted) as livestock feed ingredient
13426 : 1992	Animal feeds and feeding stuffs — Methods of sampling for aflatoxin analysis
13427 : 1992	Animal feeds and feeding stuffs — Determination of aflatoxin B_1 content
13433	Animal feeds and feeding stuffs — Determination of calcium
(Part 1) : 1992	Titrimetric method
(Part 2) : 1992	Atomic absorption spectrometric method
14825 : 2000/ ISO 5985 : 1997	Animal feeds and feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Kjeldahl method
14826 : 2000/ ISO 5985 : 1978	Animal feeds and feeding stuffs — Determination of ash soluble in hydrochloric acid
14828 : 2000/ ISO 6491 : 1998	Animal feeds and feeding stuffs — Determination of total phosphorus content — Spectrophotometric
14830 : 2000	Animal feeds and feeding stuffs — Determination of moisture content
15120 : 2002/ ISO 14565 : 2000	Animal feeding stuffs — Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc — Method using atomic absorption spectrometry
15121 : 2002/ ISO 6869 : 2000	Animal feeding stuffs — Determination of Vitamin A content — Method using high performance liquid chromatography

ANNEX B

(*Clause* 4.1.1)

INGREDIENTS FOR CHICKEN FEEDS

B-1 In the compounding of chicken feeds a variety of ingredients is used. This Annex gives a list of such ingredients.

B-1.1 Grain and Seeds

a) *Bajra, Bajri* (*Pennisetum typhoides*)
b) *Barley* (*Hordeum vulgare*)
c) *Jowar, Cholam* (*Sorghum vulgare*)
d) Oats (*Avena sativa*)
e) Panwar (*Cassia tora*)
f) *Ragi* (*Eleusine coracana*)
g) Yellow maize (*Zea mays*)
h) Wheat (*Triticum sativum*)

B-1.2 Grain By-products

a) Maize coarse flour, maize bran, maize gluten and maize gluten feed (*see* IS 2152),
b) Rice bran or solvent extracted rice bran and polishings (*see* IS 3648 and IS 3593), and
c) Wheat bran.

B-1.3 Oilcakes and Meals

a) Copra cake, coconut cake (expeller pressed or solvent extracted) (*see* IS 2154),
b) Cottonseed oilcake (decorticated) (expeller-pressed or solvent extracted) (*see* IS 1712 and IS 3592),
c) Groundnut oilcake (expeller pressed or solvent extracted) (*see* IS 1713 and IS 3441),
d) Guar (*Cyamopsis tetragonoloba*) (*see* IS 4193),
e) Maize germ oilcake (*see* IS 2151),
f) Mustard and rapeseed cake (*see* IS 1932),
g) Sal seed meal, solvent extracted (*see* IS 7059),
h) Safflower (*Carthamus tinctorius*) cake (expeller pressed or solvent extracted) (*see* IS 2503 and IS 6242),
j) Sesamum (*Sesamum indicum orientale*) cake (expeller pressed or solvent extracted) (*see* IS 1934),
k) Soyabean (solvent extracted) (*see* IS 10657), and

m) Sunflower oilcake (decorticated or undercorticated) (*see* IS 10147 and IS 10165).

B-1.4 Animal Products

a) Blood meal (*see* IS 7060),
b) Fish meal (*see* IS 4307),
c) Liver residue,
d) Meat meal and meat scrap, and
e) Meat cum bone meal (*see* IS 5065).

B-1.5 Minerals and Vitamins

a) Bone meal (steamed),
b) Common salt (*see* IS 920),
c) Dicalcium phosphate (*see* IS 5470),
d) Limestone,
e) Oyster shells, and
f) Vitamins (mineral-stabilized).

B-1.6 Waste Material and Industrial By-products

a) Brewers' grains,
b) Dried yeast and yeast sludge,
c) Mango seed kernel (*see* IS 12829),
d) Molasses (*Khandsari* type) (*see* IS 1162),
e) *Penicillin mycelium* residue, and
f) Dried silkworm pupae.

ANNEX C

(*Clause* 4.2.1)

FEED ADDITIVES

C-0 DEFINITION

Any intentionally added ingredient not normally consumed as feed by itself, whether or nor it has a nutritive value, which affects the characteristics of feed or animal products.

C-1 FEED ADDITIVES IN POULTRY NUTRITION

The production performance of birds has improved in last few years due to many factors like improvement in genetic potential of the birds, overall improvement in other inputs provided. Broilers are growing faster and layers are producing eggs at higher rate than ever before. Such birds require adequate and good nutrition. Even after fulfilling nutrient requirements as per the prescribed standards, availability of nutrients to the extent required is sometimes difficult. With this in view use of appropriate feed additives in poultry feeding has become obligatory. These feed additives improve physical appearance, consistency, nutritive quality, shelf life, sometimes nutrient availability and texture of diets. Thus, feed additives are important, though non-nutritive in nature, to help in improving growth, production and health status of birds.

C-2 NUTRITIVE FEED ADDITIVES

Vitamins, trace minerals and amino acids are essential nutrients. However, the natural feed ingredients sometimes are not able to provide these essential elements in adequate quantity in the feed. This is because of the differences in their availability and increased requirement of birds. Hence, it is needed to supplement vitamins, trace minerals and amino acids. These are available either in pure form or are premixed.

C-3 NON-NUTRITIVE FEED ADDITIVES

They include prebiotics, probiotics, acidifiers and *p*H optimizers, antioxidants, feed enzymes, toxin binders, herbal products, antibiotic growth promoters, anticoccidials, emulsifiers, flavours, and carotenoids, etc.

C-4 PREBIOTICS

Mannan oligosaccharides (MOS) are the complex carbohydrates, which are derived from cell wall of yeast. The non-digestible MOS improve the performance and health of poultry by preventing the pathogenic bacteria adhering to gut wall and by reaching the hindgut of the bird in an undigested form by influencing hindgut fermentation towards more desirable bacteria. The substance does not only affect the non-immunogenic defense mechanism in the gastro-intestinal tract, but also functions by modulating the immunogenic protection mechanism.

C-5 PROBIOTICS

The potential pressure on removal of growth promoting antibiotics from animal and poultry feed has led to renewed interest in the use of live microbial cultures

as growth promoting agents. The probiotics are live microbial feed supplements that benefit the host by establishing a favorable intestinal microbial balance and maintaining proper gut health. They are nonpathogenic to the birds as well as human beings.

They act by following ways:

a) Adherence to intestinal mucosa thereby preventing attachment of pathogens,
b) Competition with pathogens for nutrients,
c) Stimulation of the intestinal immune response,
d) Affecting the permeability of gut and increasing the uptake of nutrients, and
e) Competitive exclusion of pathogen.

C-5.1 Commonly Used Probiotics

Lactobacilli (*L. acidophillus, L. sporogens*), Saccharomyces. (*S. cerevisiae, S. boulardii*), Streptococcus (*S. lactis, S. thermophilus*), Bacillus (*B. cereus, B. subtilis*).

C-6 ACIDIFIERS AND *p*H OPTIMIZERS

The *p*H of the gastric and intestinal tract directly affects the activity of various digestive enzymes and rate of digestion of feedstuff. Additionally, the *p*H effects the species composition of intestinal microflora and prevalence of potential pathogens. *p*H of gastro-intestinal tract affects ability of pathogens to colonize the gut. Near natural *p*H may favour the growth of E. *coli* and *Salmonella*. Lower *p*H values are more conducive to the growth of friendly bacteria like Lactobacilli.

Acidifiers are especially useful in young animal and bird feeds because they are effective against disease causing microbes in gut and are relatively inexpensive compared to all natural alternatives. A feed additive composed of organic and inorganic acids with volatile fatty acids derivatives can reduce *p*H around the pathogenic bacteria causing a bacteriostatic effect. Simultaneously, such product can provide a bactericidal effect by supplying low molecular weight acid molecules that can penetrate bacterial cell.

C-6.1 Commonly Used Acids

Formic acid, propionic acid, butyric acid, lactic acid, phosphoric acid, citric acid, scrbic acid and acetic acid.

C-7 ANTIOXIDANTS

High fat containing ingredients like fishmeal, meat meal, poultry by-products and vegetable oil and oil products are the common ingredients of poultry feed. All these ingredients are highly prone to the auto-oxidative rancidity, which has adverse effects on palatability of feed and bio-availability of nutrients. This process may destroy critical nutrients like vitamin A, D, E, and biotin and ultimately has a serious negative effect on growth performance and production. Antioxidant in turn helps in preserving the nutritive value and freshness of diet. It maintains potency of dietary energy and critical vitamins. It also protects natural pigments.

C-7.1 Commonly Used Antioxidants

Butylated Hydroxyl Toluene (BHT), Butylated Hydroxyl Anisole (BHA) and Ethoxyquin, etc. singly or in the commercially available combinations.

C-8 FEED ENZYMES

Most of poultry feed ingredients contain anti-nutritional factors like non-starch polysaccharides (NSP's) and toxicants. Poultry do not have enzyme system to the extent to utilize effectively these NSP's and to reduce anti-nutrient activity of these factors. The feed enzymes are proteins in nature, catalysts, augmenting host enzyme system, and they are target specific. Supplementing feed enzymes to poultry feed reduces negative effect of NSP's and toxicants, thereby improve feed digestibility, nutrient availability, and performance.

Enzymes act by:

a) Reducing gut viscosity,
b) Improved nutrient availability, and
c) Increased digestibility and nutrient absorption.

C-9 TOXIN BINDERS

Different adsorbent materials are used therapeutically to reduce toxicity of chemical to bio-system. Several substances like activated charcoal, alumino-silicates, bentonites, silicon and zeolites, etc, have been found beneficial in minimizing the toxic effect of feed mycotoxins. Hydrated sodium calcium alumino-silicate (HSCAS) has strong effect on toxin binding.

C-10 HERBAL PRODUCTS

Parts of many herbal plants such as bark, fruit, seed, leaf, flower, etc and their extracts are used for treating the diseases and ailments in man, animals and birds. These natural products are safe and eco-friendly. Many products like growth stimulators, liver tonics, anti-stress factors, coccidiostats and immuno-modulators are prepared from herbal plants.

C-11 ANTICOCCIDIALS

Coccidiosis is a disease of economic importance caused by Eimeria group of protozoa. Ionophores and chemical compounds are regularly used in the feed to keep the coccidiosis away resulting into economic improvement

of the flock. Commonly used ionophores are salinomycin, maduramycin, lasalocid, monensin and chemical compounds such as diclazuril, amprolium and D.O.T. etc.

C-12 EMULSIFIERS

Young animals and birds and aquatic animals like shrimp are not capable of digesting fat present in feed, efficiently. They can utilize the fat efficiently, provided, emulsifiers along with antioxidants are added in the feed. Products containing phospholipids can aid in nutrient uptake from the digestive tract. Addition of such products may in turn improve growth and feed conversion.

C-13 FLAVOURS

Flavoring agents are feed additives that are supposed to increase palatability and feed intake. There is need for flavoring agents that will help to keep up feed intake:

a) When highly unpalatable medicants are being mixed in the feed,
b) During outbreak of diseases,
c) When animals are under stress, and
d) When less palatable feedstuffs are fed either as such or being incorporated in the ration.

C-14 CAROTENOID

Carotenoids are recently gaining an importance in the field of poultry nutrition because of its growth promoting and immune stimulating effect. It also stimulates phagocytic and bacteria killing ability of neutrophils and potential macrophages. Carotenoids are also used in colouring of yolk in egg and pigmentation of meat. There are both synthetic and natural colouring agents.

C-15 RECOMMENDATIONS

The use of antibiotic growth promoters are **NOT RECOMMENDED** in poultry feed. There are various reasons for prohibiting such use. Most important is the residues of antibiotic in animal tissues and products such as egg, milk and meat. Second issue is development of bacterial resistance, nullifying the purpose for which antibiotics were recommended in the past.

To counter the non-usage of antibiotic feed supplements, use of probiotics, Prebiotics, Acidifiers and Herbal preparations need to be encouraged. This would also mean better and improved hygienic practices.

It is recommended that antibiotic growth promoter with systemic action such as *chloromphenicol, doxycycline, tetracycline, nitrofurone, furozolidone* etc, should not be used in the Poultry feed. The use of gut acting antibiotics will be tapered off and stopped in next five years.

ANNEX D

(*Clause* 4.2.2)

WATER QUALITY

D-1 Water is very essential and critical nutrient in the poultry nutrition. Its importance is always underestimated in the overall feeding management. Body contains 75 percent of water. Egg contains 65 percent water. Water is needed for transport of nutrients, removal of waste material through kidneys, various metabolic processes in the body. Water is very integral part of blood, lymph, tissue fluid and cellular fluid.

In the normal circumstances water is available from feed (10 percent), metabolic processes (20 percent) and remaining from free flowing source. Poultry must have access to the water 24 h. As a thumb rule, water consumption is double the feed consumption in case of birds at the temperature of about 18°C. It goes up by five to six times in hot weather. An abnormal increase in water consumption signals the disturbance. Water deficiency results in reduction in feed intake, drop in performance, abnormalities and death.

Water is one of the prime sources of infection because it easily gets contaminated. Water offered to poultry must be clean and free from pathogenic microorganisms. It should be clear, colourless, and odourless without any sediment. An ideal *p*H of water is 6.0.

Apart form being contaminated by microbes, salinity of water is a major problem for poultry. Excessive salinity can upset the animals water balance and may cause health problems and death.

If water is contaminated by microbes, use of various water sanitizers is advised. These include, bleaching powder, potassium permanganate, acidifiers, iodine, sodium hypo-chloride, dimethyl alkyl benzalkonium chloride. Water treatment may be carried out in consultation with expert in the field is best recommended.

Limits of Toxic Substances for Drinking Water for Livestock

No.	*Constituent*	*Upper Limit* mg/litre	*Method of Test*
i)	Aluminum (Al)	5.0	A.O.A.C.
ii)	Arsenic (As)	0.2	A.O.A.C.
iii)	Boron (B)	5.0	A.O.A.C.
iv)	Cadmium (Cd)	0.05	A.O.A.C.
v)	Lead (Pb)	0.1	A.O.A.C.
vi)	Mercury (Hg)	0.01	A.O.A.C.
vii)	Nitrate + Nitrite ($NO_3 - N + NO_1 - N$)	100.0	A.O.A.C.
viii)	Chromium (Cr)	1.0	A.O.A.C.
ix)	Cobalt (Co)	1.0	A.O.A.C.
x)	Copper (Cu)	0.5	A.O.A.C.
xi)	Fluorine (Fl)	2.0	A.O.A.C.
xii)	Nitrite ($NO_2 - N$)	10.0	A.O.A.C.
xiii)	Selenium (Se)	0.05	A.O.A.C.
xiv)	Vanadium (V)	0.10	A.O.A.C.
xv)	Zinc (Zn)	24.0	A.O.A.C.

ANNEX E

(*Clause* 6)

SAMPLING OF CRITERION FOR CONFORMITY OF POULTRY FEEDS

E-1 GENERAL REQUIREMENTS OF SAMPLING

E-1.0 In drawing, preparing, storing and handling samples, care shall be taken that the properties are not affected. The following precautions and directions shall be observed.

E-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

E-1.2 The sampling instrument shall be clean and dry when used.

E-1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers for samples from adventitious contamination.

E-1.4 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely filled by the sample.

E-1.5 Each container shall be scaled air-tight with a stopper or a suitable closer after filling in such a way that it is not possible to open and reseal it without detection, and marked with full details of sampling, date of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

E-1.6 Samples shall be stored in such a manner that there is no deterioration of the material.

E-1.7 Sampling shall be done by a person agreed to between the purchaser and the vendor and if desired by any of them in the presence of the purchaser (or his representative) and the vendor (or his representative).

E-2 SCALE OF SAMPLING

E-2.1 Lot

The quantity of poultry feed of a particular type produced under relatively similar conditions in a day shall constitute a lot.

NOTE — Relatively similar conditions would mean the use of raw materials having insignificant variations, similar conditions of manufacture, etc.

E-2.1.1 Samples shall be tested for each lot for ascertaining conformity of the material to the requirements of the specifications.

E-2.2 The number of bags to be selected from the lot shall depend on the size of the lot and shall be as given below:

Lot Size	*Number of Bags to be Selected*
(1)	(2)
Up to 50	5
51-100	8
101-300	13
301-500	22
501 and above	32

E-2.3 The bags shall be chosen at random from the lot and for this purpose a random number table as agreed to between the purchaser and the vendor shall be used (*see* IS 4905). If such a table is not available, the following procedure shall be adopted:

Starting from any bag count 1, 2, 3..... etc, up to *r* and so on in a systematic manner and withdraw the *r*th bag; *r* being the integral part of *N/n*; where *N* is the total number of bags in the lot, and *n* the number of bags to be as given in **E-2.2**.

E-3 TEST SAMPLES AND REFEREE SAMPLES

E-3.1 Preparation of Individual Samples

Draw with an appropriate sampling instrument equal quantities of the material from the top, bottom and the sides of each bag selected according to Table 4. The total quantity of the material drawn from each bag shall be not less than 1 kg. Mix all the portions of the material drawn from the same bag thoroughly. Take out about 1.5 kg of material and divide into three parts. Each portion thus obtained, shall constitute the test sample representing that particular bag and shall be transferred immediately to clean and dry sample containers and sealed air-tight. These shall be labelled with particulars given under **E-1.5**. The individual samples obtained as above shall be formed into three sets in such a way that each set has a test sample representing each bag selected. One of the sets shall be for the purchaser, another for the vendor and third for the referee.

E-3.2 Preparation of Composite Sample

From the mixed material from each selected bag remaining after the individual samples have been taken, equal quantities of material from each bag shall be taken and mixed up together so as to form a composite sample weighing not less than 1.5 kg. This composite sample shall be divided into three equal parts and transferred to clean and dry containers and labelled with the particulars given under **E-1.5** and sealed air-tight. One of these samples shall be for the purchaser, another for the vendor and third for the referee.

E-3.3 Referee Sample

Referee samples shall consist of a set of test samples (*see* **E-3.1**) and composite samples (*see* **E-3.2**), and shall bear the seals of the purchaser and the vendor and shall be kept at a place agreed to between the two.

E-4 TESTING OF SAMPLES

E-4.1 Test for crude protein and crude fibre shall be conducted individually on each of the samples constituting a set of test sample (*see* **E-3.1**).

E-4.2 Test for the remaining characteristics, prescribed in Table 1 shall be conducted on the composite sample (*see* **E-3.2**).

E-5 CRITERION FOR CONFORMITY

A lot shall be considered as conforming to the specification when:

a) Each of the test results for crude protein and crude fibre satisfies the requirements as specified in Table 1, and

b) The test results on the composite sample satisfy the other requirements specified in Table 1.

ANNEX F

[*Table* 2, *Sl No.* (iii)]

DETERMINATION OF AVAILABLE PHOSPHORUS

F-1 REAGENTS

F-1.1 Aminonaphtholsulphonic Acid — Place 195 ml of 15 percent sodium bisulphite solution (*see* **F-1.1.1**) in a glass stoppered cylinder. Add to it 0.5 g of 1, 2, 4-aminonaphtholsulphonic acid and 5 ml of 20 percent solution (*see* **F-1.1.2**). Stopper the cylinder and shake well to dissolve the powder. If the powder is not dissolved completely add more sodium sulphite solution, 1 ml at a time, with shaking but avoid excess. Transfer the solution to a brown-glass bottle and store in the cold. The solution, if stored as described, may be used for about four weeks.

F-1.1.1 *Sodium Bisulphite Solution*, 15 percent (*m/v*). Weigh accurately 30 g of sodium bisulphite in a beaker and add 200 ml of water. Stir to dissolve, and if the solution is turbid, allow to stand well, stoppered for several days and then filter. Keep the solution well stoppered.

F-1.1.2 *Sodium Sulphite Solution* — Dissolve 20 g of anhydrous sodium sulphite in water and dilute to

100 ml, if necessary filter the solution. Keep the solution well stoppered.

F-1.2 Calcium Chloride Solution, 10 percent saturated with calcium hydroxide at *p*H 8.8.

F-1.3 Calcium Chloride Solution, 20 percent (*m/v*).

F-1.4 Hydrochloric Acid, dilute.

F-1.5 Molybdate I — Dissolve 25 g of reagent grade ammonium molybdate in about 200 ml of water. Place in one litre graduated flask, 500 ml of 10 N sulphuric acid (*see* **F-1.10**) and add to it the molybdate solution. Dilute to one litre with water. Mix well.

F-1.6 Molybdate II — Dissolve 25 g of reagent grade ammonium molybdate in about 200 ml of water. Place in one litre graduated flask, 800 ml of 10 N sulphuric acid (*see* **F-1.10**) and add to it the molybdate solution. Dilute to one litre with water. Mix well.

F-1.7 Phenolphthalein Indicator Solution — Dissolve 0.1 g of phenolphthalein in 100 ml of 95 percent (*m/v*) ethyl alcohol.

F-1.8 Sodium Hydroxide Solution, saturated.

F-1.9 Standard Phosphate Solution — Weigh exactly 0.351 g of pure dry monopotassium phosphate and dissolve in water. Transfer quantitatively to a one litre graduated flask. Add to it 10 ml of 10 N sulphuric acid (*see* **F-1.10**) and make up the volume to the mark. Shake thoroughly. This solution contains 0.4 mg of phosphorus in every 5 ml of the solution.

F-1.10 Sulphuric Acid, 10 N. Add carefully 450 ml of concentrated sulphuric acid to 500 ml of water. To check, dilute 10 ml of this solution to 100 ml in graduated flask, mix and titrate a 10 ml portion of this solution with standard 1 N sodium hydroxide solution. From the titration results, adjust, if necessary, the normality of the original solution to make it exactly 10 N.

F-1.11 Trichloroacetic Acid, 5 percent (*m/v*). Dissolve 5 g of the reagent grade trichloroacetic acid in water and dilute to 100 ml.

F-2 APPARATUS

F-2.1 Colorimeter

F-3 PROCEDURE

F-3.1 Weigh accurately about 20 g of the ground material and transfer it to a 250 ml beaker. Add 100 ml of trichloroacetic acid (maintained at about 5°C) and stir occasionally for 15 min. Allow it to stand for 2 h.

F-3.2 Transfer the contents to a 250 ml graduated flask and make up the volume to the mark with trichloroacetic acid (maintained at about 5°C). Stir the contents of the flask thoroughly and allow to stand for 30 min. Filter about 120 ml of the supernatant liquid and transfer 100 ml of the filtrate to a 250 ml beaker. Neutralize with the sodium hydroxide solution using phenolphthalein as the indicator. Add to it 2 ml of calcium chloride solution (*see* **F-1.2**) and allow it to stand at room temperature for 10 min. Centrifuge the precipitate and wash with a small volume of water containing the calcium chloride solution (*see* **F-1.3**). Filter and wash. Place the funnel containing the filter paper and the precipitate on an empty 100 ml graduated flask. Dissolve the precipitate with dilute hydrochloric acid, wash the filter paper, and then make up the volume of filtrate to the mark.

F-3.3 Transfer 5 ml of the filtrate to a 10 ml graduated cylinder and add to it 1 ml of the molybdate II reagent (*see* **F-1.6**). Shake thoroughly and add 0.4 ml of the aminonaphtholsulphonic acid reagent and mix again. Make up the volume to the 10-ml mark with water, mix, and allow the contents to stand for 5 min.

F-3.4 For taking the colorimetric measurement, compare in the colorimeter against a standard prepared at the same time as given below:

> Transfer 5 ml of the standard phosphate solution containing 0.4 mg phosphorus (*see* **F-1.9**) to a 100 ml graduated flask and add 50 ml of water. Add 10 ml of molybdate I reagent (*see* **F-1.5**). Mix thoroughly and add 4 ml of aminonaphthol-sulphonic acid reagent. Dilute with water to the 100 ml mark, mix well and allow the contents of the flask to stand for 5 min. Compare the standard against itself in the colorimeter before taking a reading of the unknown solution. If the colour of the unknown is particularly strong, repeat the reading of the unknown a few minutes later, to make sure that the maximum colour development has taken place.

F-3.5 Calculate the percentage of the available phosphorus in the material from reading the colorimeter.

ANNEX G

[*Table* 2, *Sl No.* (iv)]

DETERMINATION OF LYSINE CONTENT

G-1 PRINCIPLE OF THE METHOD

Lysine is estimated by the ion-exchange chromatographic technique.

G-2 REAGENTS

G-2.1 Sodium Citrate Buffer (*p*H 5.28 ± 0.02) — Dissolve 98.2 g of citric acid (analytical reagent), 57.6 g of sodium hydroxide in 27.2 ml of concentrated hydrochloric acid and dilute to four litres. Adjust the *p*H of the solution to 2.20 ± 0.03 and then preserve by adding 0.1 ml of caprylic alcohol (0.01 percent).

G-2.2 Sodium Citrate Buffer (*p*H 2.20 ± 0.03) — Dissolve 105 g of citric acid and 42 g of sodium hydroxide in 80 ml of concentrated hydrochloric acid. Mix and dilute to 5.0 litres. Adjust the *p*H to 2.20 ± 0.03 and then add the preservative as in **G-2.1**.

G-2.3 Hydrindantin — Dissolve 20 g of ninhydrin in 500 ml of distilled water maintained at 90°C. To this add by constant stirring a solution of ascorbic acid (20 percent) maintained at 40°C. Crystalline hydrindantin (reduced form of ninhydrin) is precipitated and the crystallization is allowed to proceed for 30 min at room temperature. Filter and wash the hydrindantin free of ascrobic acid by repeated washings and dry under vacuum by keeping overnight, over phosphorus pentoxide. Store hydrindantin in dark bottle for further use.

G-2.4 Sodium Acetate Buffer (*p*H 5.5) — Dissolve 544 g of sodium acetate in 700 ml of distilled water. To this solution add 100 ml of glacial acetic acid and make the volume up to one litre. Adjust the *p*H of 5.5 with the addition of alkali or acetic acid.

G-2.5 Methyl Cellosolve (Ethylene-Glycol-Monoethyl Ether), peroxide free. To make methyl cellosolve free of peroxide, distil at 180°C before use.

G-2.6 Ninhydrin-Hydrindantin Reagent — Dissolve 2 g of ninhydrin and 300 mg of hydrindantin in 75 ml of methyl cellosolve. Then add 25 ml of sodium acetate buffer solution (*see* **G-2.4**) and thoroughly mix. This reagent should be prepared for use. Reagent, when discoloured, should not be used.

G-3 PROCEDURE

G-3.1 Preparation and Hydrolysis of Sample

Take 100 mg of finely ground fat-free material and hydrolyze with 5 ml of standard hydrochloric acid in scaled Pyrex tubes at 110°C for 18 to 24 h. On hydrolysis, make the volume to 10 ml with distilled water. Take an aliquot of 2 ml and evaporate to near dryness to remove the excess acid. Using sodium citrate buffer (*p*H 2.20), make the volume to 10 ml. In case the sample is not used immediately, the hydrolysates are kept under refrigerated condition.

G-3.2 Preparation and Operation of the Column

The chromatographic column is poured with slurry of the treated resin, Amberlite IR 120 (300 to 400 mesh), in standard sodium hydroxide solution. A total length of 16 cm is poured in 3 to 4 sections. The first few centimetres of the column are allowed to settle by gravity and the rest is packed with a pressure of 1.0 to 1.5 kg/cm^2 from an air compressor connected to the column. When the required length of the resin column is poured and packed, the supernatant alkali is removed and the resin equilibrated with sodium citrate buffer (*p*H 5.28 ± 0.02).

The resin column is brought to a constant temperature (50 ± 1°C) by circulating warm water from a thermostatic water bath. 2 ml of the protein hydrolysate is added on top of the column without disturbing the top layer and a pressure of 1 to 1.5 kg/cm^2 is applied. As the hydrolysate enters the top layers of the column, two to three washings with 0.5 ml portions of citrate buffer of *p*H 5.28 are given. A separating funnel filled with the buffer is fitted to the column and pressure applied to maintain an effluent flow rate of 24 ml/h. The collection of the effluent is started leaving the first few tubes and subsequent 2 ml fractions are collected by means of a 2 ml syphon. A total of 24 tubes are collected. The first 9 or 10 tubes, which consist of neutral and acidic amino acids, are discarded. To each of the latter 12 tubes exactly 1 ml of the ninhydrin-hydrindantin reagent is added. A reagent blank is similarly treated. The tubes are heated in vigorously boiling water bath at 100°C for exactly 15 min, removed from the bath and immediately chilled in an ice bath. After making the solution to volume, the colour is read in a spectrophotometer at 590 nm against a reagent blank.

G-3.3 Standard Curve

Lysine standard solutions of 0.05 to 0.25 mg/ml concentrations are prepared and the colour developed as in the unknown solutions. A standard graph is plotted against which the concentration of the unknown may be read.

ANNEX H

[*Table* 2, *Sl No.* (iv)]

DETERMINATION OF METHIONINE CONTENT

H-1 REAGENTS

H-1.1 Standard Sodium Hydroxide Solution, 5 N.

H-1.2 Sodium Nitroprusside, 10 percent in water. The solution is prepared afresh every time before use.

H-1.3 Glycine, 3 percent solution in water.

H-1.4 Phosphoric Acid, 85 percent.

H-1.5 Hydrochloric Acid, 6 N.

H-1.6 Phosphotungstic Acid

H-2 APPARATUS

H-2.1 Spectrophotometer

H-3 PROCEDURE

H-3.1 Take 100 mg of finely ground fat-free material and hydrolyze with 5 ml standard hydrochloric acid in sealed Pyrex tubes at 110°C for 18 to 24 h. At the completion of hydrolysis, open the tubes and transfer the contents to a 10 ml volumetric flask with repeated washings. Add 50 mg of activated charcoal (previously treated with standard hydrochloric acid) and 50 mg of phosphotungstic acid to the flask. Mix well and make the volume to 10 ml with distilled water and filter. Take 2 ml of acid hydrolysate and add 2 ml of water, 1 ml of standard sodium hydroxide solution, 1 ml of standard methionine solution and 0.1 ml of sodium nitroprusside solution. Shake the mixture thoroughly for 10 min in a mechanical shaker. Subsequently, add 2 ml of glycine solution and again shake for 10 min and read the colour at 540 nm in a spectrophotometer.

H-3.2 Prepare standard solution containing 200 to 1 000 µg/ml methionine. Treat this solution similarly as in **H-3.1** and establish a standard curve. Run an identical reagent blank. The amount of methionine is expressed as gram per 16 g of nitrogen.

ANNEX J

[*Table* 2, *Sl No.* (vii)]

DETERMINATION OF METABOLIZABLE ENERGY (ME)

J-1 APPARATUS

J-1.1 Brooder, battery or floor type.

J-1.2 Glass Bottles, stoppered, wide-mouthed.

J-2 REAGENTS

J-2.1 Acetic Acid, 2 percent.

J-2.2 Sulphuric Acid, 5 percent.

J-3 PROCEDURE

J-3.1 Place 25 three weeks old healthy chicks (White Leghorn or Rhode Island Red) in a brooder and rear them on the experimental feed for an 8-day acclimatization period. Then on the second day of the fifth week, give the chicks the requisite amount of accurately weighed experimental feed at a fixed hour in the morning. Simultaneously, spread the polyethylene sheets on the faeces trays for the collection of excreta. Collect a representative sample of feed for dry matter percentage and proximate analysis. Next day at the same hour collect the remaining feed and the faeces excreted and weigh them. A representative sample of the remaining feed is again collected for dry matter percentage. The difference in dry mass of feed offered and the remaining feed gives the amount of dry matter consumed during 24 h. Collect the aliquots from excreta, after mixing it well, for dry matter and nitrogen estimation separately (one-twentieth and one-hundredth parts respectively) in wide-mouthed and glass-stoppered bottles, and keep them in a refrigerator. For nitrogen estimation, samples in duplicate shall be preserved in 5 percent sulphuric acid.

NOTE — The age of the birds during the collection period may vary from 5 to 10 weeks, but this does not affect the ME value significantly.

J-3.2 Repeat the same procedure on the next two alternate days and pool together the three-day samples of excreta for the analysis. For dry matter estimation

add about 10 ml of 2 percent acetic acid for every 50 g of the excreta and dry it in an oven at about 80°C till constant mass is obtained. Find out the total dry matter voided in three days.

J-3.3 Analyze the samples of feed and the excreta for their crude protein, ether extract, total ash and total carbohydrate content. Calculate on dry matter basis.

J-4 CALCULATION

Calculate gross calories in feed and excreta. The calorific values of crude protein, ether extract and total carbohydrates are 5.65, 9.40 and 4.15 cal/g, respectively.

$$\text{Metabolizable energy per kg feed} = (E_{diet} - E_{excreta} - N \times 8.22 \times 1\,000)$$

where

E_{diet} = gross calories per g of feed (dry matter),

$E_{excreta}$ = gross calories in excreta per g of the feed (dry matter) consumed, and

N = nitrogen retention in g per g of feed (dry matter) consumed. This is obtained by subtracting total nitrogen excreted from the total nitrogen consumed per gram of the feed (dry matter).

ANNEX K

[*Table* 2, *Sl No.* (viii)]

DETERMINATION OF AFLATOXIN B_1 (ppb)

K-1 REAGENTS

K-1.1 Water, complying with at least Grade 3 in accordance with relevant Indian Standard.

K-1.2 Toluene

K-1.3 Chloroform, HPLC grade.

K-1.4 Potassium Hydroxide Solution (KOH)

K-1.5 Light Petroleum Ether, boiling range 40° to 60°C.

K-1.6 Sodium Sulphate (Na_2SO_4), anhydrous.

K-1.7 *n*-Hexane

K-1.8 Acetonitrile

K-1.9 Glacial Acetic Acid

K-1.10 Diethyl Ether

K-1.11 Silica Gel

K-1.12 Inert Gas, for example nitrogen.

K-1.13 Aflatoxin B_1 Standard

K-2 APPARATUS

High performance liquid chromatograph, consisting of the following:

a) *Pump*, set to deliver a constant eluent volume flow rate of 0.5 ml/min.

b) HPLC injection device.

c) *Column*, length 200 mm, internal diameter 8 mm, packed with a stationary phase consisting of octadecyl (C_{18}) groups bonded to silica.

d) *Detector*, fluorescence.

e) Boiling water bath.

f) *Rotary vacuum evaporator*, with water bath at 40°C.

g) Mechanical shaker.

K-3 PREPARATION OF TEST SAMPLE

Weigh 50 g sample in a conical flask. Add 150 ml of petroleum ether and keep overnight. Filter through ordinary filter paper and add 250 ml of chloroform. Well mix the contents for 30 min in mechanical shaker. Filter the contents through ordinary filter paper with anhydrous Na_2SO_4 bed. Extract ready for purification through column chromatography.

K-4 PREPARATION OF COLUMN CHROMATOGRAPHY

Take 300 mm × 10 mm ID of glass column. Put some glass wool inside the column and remove air bubbles by using 10 ml of chloroform. Add 1 g of anhydrous Na_2SO_4. Add 2 g of slurry of activated silica gel into the column. (Silica gel dried for 1 h at 105°C and add 1 ml/gm chloroform to the dried silica gel. Keep it for 15 h.) Add stabilized chloroform into the column. It shall be 3 cm above the silica gel. Again add 2 g of anhydrous Na_2SO_4 to form a bed. Remove air bubbles by using chloroform, which shall be above the bed.

K-5 SAMPLE PURIFICATION

Elute 75 ml extract of aflatoxin through the column slowly and collect it. Pour one by one, 25 ml of each of toluene: glacial acetic acid (9:10), *n*-hexane and acetonitrile: diethyl ether: *n*-hexane (10:30:60 *v/v*) on the column and discard it. Finally elute aflatoxin with 50 ml of chloroform: acetone (4:1 *v/v*), mix and collect it in a 250 ml round bottom flask. Concentrate the extract in a rotary vacuum evaporator. Dry the concentrate extract on a water bath under a stream of nitrogen gas at 50°C. Reconstitute the dried extract with mobile phase and inject it in HPLC column.

K-6 HPLC CONDITIONS

Temperature	: 30°C
Flow rate	: 0.5 ml/min
Detector	: Fluorescence
Wave length	: Ex 369 nm, Em 430 nm
Runtime	: 5 min
Maximum pressure	: 300 kg/cm^2
Column	: C_{18} (200 mm × 8 mm)
Mobile phase	: Acetonitrile: water (65:35)

K-7 CALCULATION

$$\text{Aflatoxin } B_1(\mu g/l) = \frac{B_{af} \times V_{ext}}{V_B \times W \times V_f / 250}$$

B_{af} = concentration of aflatoxin B_1 as determined from the calibration curve (ng);

V_B = volume of sample extract injected, in microlitres;

V_{ext} = volume in which dried extract was dissolved;

W = weight of the sample;

V_f = volume of filtrate taken in the column for elution, in ml; and

250 = volume of chloroform used in the extraction, in ml.

ANNEX L

[*Table* 3, *Sl No.* (viii)]

DETERMINATION OF VITAMIN D_3

L-1 REAGENTS

L-1.1 Water, complying with at least grade 3 in accordance with relevant Indian Standard.

L-1.2 Ethanol, $w(C_2H_5OH)$ = 95 per cent (by volume), or equivalent methylated spirit.

L-1.3 Methanol, HPLC grade.

L-1.4 Methyl Chloride, HPLC grade.

L-1.5 Phosphate Buffer, 0.1 M.

L-1.6 Diethyl Ether

L-1.7 Acetone, HPLC grade.

L-1.8 Acetonitrile

L-1.9 Inert Gas, for example, nitrogen.

L-1.10 Vitamin D_3 Standard Substances

L-2 APPARATUS

High performance liquid chromatograph, consisting of the following:

a) Pump, set to deliver a constant eluent volume flow rate of 2.0 ml/min.

b) HPLC injection device.

c) Column, length 150 mm, internal diameter 4 mm, packed with a stationary phase consisting of octadecyl (C_{18}) groups bonded to silica.

d) Detector, allowing the measurement of ultraviolet radiation at 265 nm and equipped with integrator/data handling system.

e) UV (or UV-Visible) spectrometer, capable of measuring absorbance at the different wavelength and equipped with quartz cells of 10 mm path length.

f) Boiling water bath.

g) Rotary vacuum evaporator, with water bath at 40°C.

h) Extraction apparatus.

j) Membrane filter, 0.22 µm pore size, for filtration of mobile phase and sample test solutions.

L-3 PREPARATION OF TEST SAMPLE

Take 1.0 g feed sample in amber colour vial. Add 5 ml of diethyl ether. Shake vigorously and keep vial in a beaker containing acetone in freezer until lower portion is freezed. Take out supernatant from the vial into another vial. Add 4 ml (methyl chloride: methanol, 3:1) in each vial and collect the supernatant. Take out supernatant in another vial. Add 5 ml of 0.1 M-phosphate buffer in each vial and collect the supernatant. Dry it in water bath at 34-36°C or in oven. Complete drying under nitrogen gas. Reconstitute in mobile phase. Filter through 0.22 µ filter paper. Inject known quantity in HPLC column.

L-4 HPLC CONDITIONS

Temperature	: 25° C
Flow rate	: 2.0 ml/min
Detector	: UV
Wave length	: 265 nm
Runtime	: 10 min
Maximum pressure	: 400 kg/cm^2
Column	: C_{18} (150 mm × 4 mm)
Mobile phase	: Acetonitrile (100 percent)

L-5 CALCULATION

$$\text{Vitamin } D_3 (\mu g/g) = \frac{V_e \times S_A \times S_{dC} \times \text{Purity of vitamin } D_3}{V_i \times S_{dA}}$$

where

V_e = volume in which the dried sample was dissolved,

S_A = sample area from peak,

S_{dC} = standard concentration (Vitamin D_3),

V_i = volume injected, and

S_{dA} = standard area from the peak.

ANNEX M

[*Table* 3, *Sl No.* (xvii)]

DETERMINATION OF VITAMIN E

M-1 REAGENTS

M-1.1 Water, complying with at least Grade 3 in accordance with relevant Indian Standard.

M-1.2 Ethanol, *w* (C_3H_5OH) = 95 percent by volume, or equivalent industrial methylated spirit.

M-1.3 Methanol, HPLC grade.

M-1.4 Potassium Hydroxide Solution (KOH)

M-1.5 Light Petroleum Ether, boiling range 40° to 60°C.

M-1.6 Sodium Sulphate (Na_2SO_4), anhydrous.

M-1.7 Tetrahydrofuran

M-1.8 Acetonitrile

M-1.9 Inert Gas, nitrogen.

M-1.10 Vitamin E Standard Substances

M-2 APPARATUS

High performance liquid chromatograph, consisting of the following:

a) *Pump,* set to deliver a constant eluent volume flow rate of 1.5 ml/min;

b) HPLC injection device;

c) *Column,* length 150 mm, internal diameter 4 mm, packed with a stationary phase consisting of octadecyl (C_{18}) groups bonded to silica;

d) *Detector,* allowing the measurement of ultraviolet radiation at 290 nm and equipped with integrator/data handling system;

e) *UV (or UV-Visible) spectrometer,* capable of measuring absorbance at the different wavelength and equipped with quartz cells of 10 mm path length;

f) Boiling water bath;

g) Rotary vacuum evaporator, with water bath at 40°C;

h) Extraction apparatus; and

j) Membrane filter, 0.22 µm pore size, for filtration of mobile phase and sample test solutions.

M-3 PREPARATION OF TEST SAMPLE

Take 1.0 g feed sample in amber colour vial. Saponify with 10 ml of 95 percent ethanol and 2 ml of 60 percent KOH. Keep the vial in hot water bath till one or two bubbles appear. Keep it in ice bath for 5 min. Add 10 ml of petroleum ether (BP 40-60°C). Shake it on water bath shaker (35-40°C) for 15 min. Remove ether portion in another tube. Repeat the extraction with petroleum ether three times. Treat the pooled ether extract with 0.5 N KOH (10 ml). Give three washings with distilled water to remove KOH and collect the upper portion of petroleum ether. Pass the ether extract through anhydrous Na_2SO_4 or phase separator filter paper. Dry ether extract at 40-50°C under nitrogen gas. Reconstitute in mobile phase. Filter through 0.22 μ filter paper. Inject known quantity in HPLC column.

M-4 HPLC CONDITIONS

Temperature	:	17°C
Flow rate	:	1.5 ml/min
Detector	:	UV
Wave length	:	290 nm
Runtime	:	6 min
Maximum pressure	:	350 kg/cm^2
Column	:	C_{18} (150 mm × 4 mm)
Mobile phase	:	Acetonitrile: tetrahydrofuran: water (47:42:11)

M-5 CALCULATION

$$\text{Vitamin E } (\mu g/g) = \frac{V_e \times S_A \times S_{dC} \times \text{Purity of vitamin E}}{V_i \times S_{dA}}$$

V_e = volume in which the dried sample was dissolved,

S_A = sample area from peak,

S_{dC} = standard concentration (Vitamin E),

V_i = volume injected, and

S_{dA} = standard area from the peak.

ANNEX N

(Foreword)

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