

QUALITY AND SAFETY OF ANIMAL FEEDS IN INDIA

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The efficiency of feed utilization in the livestock and poultry birds and the development of feed industry of a country is dependent upon the quality of feeds. The quality of compounded animal feeds is based on the quality of its constituents i.e. the raw material (cereals, cereal by products, oilseed meals, marine feeds, agro industrial by products), used to formulate the ratio.

Feed quality has been defined as “any of the features that makes something what it is” and “the degree of excellence which a thing possesses.” A quality feed would supply all nutrients in adequate quantity and high digestibility and ingestibility.

The Food and Agriculture Organization (FAO) of the United Nations has reported that by 2020, the developing countries should be producing roughly half of the milk with India leading in the output. (Anon. 2003a). Further, in most developing countries, the per capita milk production has kept pace with the population growth. The dairy production also expanded along with its consumption which is particularly true for India. This was made possible due to the joint efforts of the farmers, nutritionists and dairy scientists, resulting in a significant increase in the milk production in India (84 MT as annum). In this venture, local feed manufacturers and agro-pharmaceutical companies also helped, especially by developing protein, mineral and vitamin rich products.

In India, they are also used in compound as well as home made feeds. In oil seed cakes category, soyabean, groundnut, mustard, linseed, sesam and sunflower are used in cattle and poultry feeds. Sometimes, other cakes such as cottonseed and copra are also used as main ingredients. Most compounded feeds contain limited amount of grains and oilseed cakes. On the contrary, over-heating of soybeans during the roasting and oil removing steps could reduce the nutritional quality, and the steam treatment could increase the moisture and rancidity in rice polish. Besides, deterioration of feed during storage in the godown causes rancidity resulting in the peroxidation and polymerization of unsaturated fatty acids and free fatty acids to aldehydes. The aldehydes in turn react with some free acid groups of the protein, resulting in the decreased nutritional value of the finished feed.

In India the quality control is regulated by to a statutory body Bureau of Indian Standards (BIS). It was established under BIS Act, 1986. Earlier, Indian standards Institute was regulating the quality control of various feed commodities. The objectives of BIS are as follows:

1. Harmonious development of the activities for standardization of various commodities.
2. Marking
3. Quality certification of goods
4. Attending to the connected methods

Bureau has set up subcommittees for the standardization of different types of commodities. A sub-committee on animal feeds called Animal Feeds Sectional Committee has been specifically set up to check the quality of animal feeds and feed ingredients. The members of animal feeds sectional committee are the eminent nutritionist taken from the :

1. Indian Council of Agricultural Research (ICAR) institutes
2. State Agricultural Universities
3. Feed Industry
4. Government departments having specialization in Animal Nutrition
5. Feed Technologist concerned with Animal Husbandry Activities.

The objectives to constitute the sectional committees are:

1. To describe the feeds accurately
2. To lay down standards on feed ingredients
3. To lay down standards for compounded feed formulations and mineral mixtures for cattle, poultry, pigs, laboratory animals, etc.

The standards laid down by the sub-committees are published as BIS specification .The approved published standards are revised from time to time. In India, BIS is responsible for publishing various methods of analysis of nutrients and anti nutritional factors present in animal feed as BIS standards/specifications.

The Government of India is empowered with registration act on the Agricultural produce (Grading and Marketing), known as 'AGMARK' standards to fix quality standards and prescribe terms and conditions for using the seal, 'AGMARK'.

Quality Control

The objective of quality control of feedstuffs is to ensure that a consumer should obtain feeds that are unadulterated, true to their nature and produce desired results. Quality control is therefore, defined as the maintenance of quality at levels and tolerances acceptable to the buyer while minimizing the cost of processing.

Quality commitment and points to evaluate

To organized an in-plant quality control program, an overview of the total operation is the primary consideration; and the development of a quality control manual is logical first step as a useful guide to action. As an employee training tool, and as a reference for all company personnel .A typical quality control manual will usually the following:

- An index or outline of content
- A statement of the company's quality control philosophy
- In-plant quality control supervisory and operator duties and responsibilities
- Sampling practices and procedures for ingredients and finished products
- A suggested ingredient assay schedule
- Laboratory report including interpretation as to their use
- Regulation and compliance (Good Manufacturing Practices)
- Production record keeping and procedures
- Package weight control, labeling, and coding
- Complaint procedures
- Product recall procedures
- Rework material guidelines
- Housekeeping (sanitation)requirements
- Ingredient purchasing specifications
- Warehousing and pest control practices
- Shelf-life and finished product turnover standards
- Guidelines for medicated feed manufacturing and handling
- Plant formula guidelines/standard operating practices for the handling of new and old formulas.
- Employee training in quality control
- In-process sampling, testing method, and test equipment for particle size reduction, batching and mixing, pellet quality , etc
- Maintenance practices and responsibilities
- Assignment of one person for total coordination of the program. The person should be given clear authority to articulate conditions and problems to management and should not be restricted in that by purchasing , production, sales, or any other person.
- or function
- All plant personnel , including delivery personnel , should be involved in the program and trained to perform their , individual quality control duties.
- All quality control stations-receiving; the various processing locations such as grinding, mixing ,pelting, and others; and bulk load out-should be provided with the necessary test equipment, forms for recording test results, sample bags, and other supplies.
- Periodic, routine compliance inspections should be conducted by appropriate management personnel using checklist to ascertain that the

company's quality commitment standard are being met; and the results of those inspections should be shared with all levels of management as well as with plant and truck fleet employees.

Common Adulterants in Feeds and Fodders

Adulteration is defined as the admixture of a pure substance with some cheaper and low quality substance. It is done intentionally usually to make money. In costly feed ingredients like oil seed cakes and feeds of animal origin like fish meal, adulteration is done by spraying urea in order to raise their protein content. However, sometimes brans, molasses are also added. Besides urea, oilseed cakes are adulterated with husk, non edible oilseed cakes.

Table 1. Common Adulterants of Different Feed Ingredients.

Feed ingredient	Adulterant
Groundnut cake	Groundnut husk; urea, non-edible oil cakes
Mustard cake	<i>Argimona maxicana</i> seeds, fibrous feed ingredients, urea.
Soybean meal	Urea, raw soybean
De oiled rice bran, wheat bran	Ground rice husk, saw dust.
Fish meal	Common salt, urea, sand
Mineral mixture	Common salt, marble powder, sand, lime stone
Molasses	Water
Maize	Cobs
Rice kani	Marble, grit

Quality Control of Feeds and feed ingredients

Quality control specifications of various feed ingredients and compound feeds laid down by BIS ensures to meet the minimum contract specifications, suitable for inclusion in the compounded feeds and indicating the maximum proportions of inclusion of feed stuffs.

Sampling of feeds: In India, BIS has laid down the following procedure and precautions for collecting the samples for analysis.

General requirements:

- In drawing, preparing, storing and handling samples, care should be taken that the properties of feeds are not affected.
- Take samples at a protected place not exposed to damp air, dust or soot.
- The sampling instrument shall be clean, dry and sterile when used.

Table 2. Quality Control of feed ingredients

<p>Ingredient Quality (Qualitative)</p>	<p>Physical characteristics (analyst's skills): Color, Texture, Odor and Taste, Particle size (screen analysis), shape, Adulteration, damage and deterioration, bulk density, storage pests, cfeaal material, hairs etc, spot chemical tests.,</p>
<p>Ingredient Quality (Quantitative)</p>	<p>Chemical analysis: Moisture, CP, CF, EE, NFE, ash, Acid insoluble ash (silica or sand), salts, free fatty acids, biogenic amins urea, and NPN, amino acids.</p> <p>Anti-nutritional factor:</p> <p>Extrinsic (contaminants): mycotoxins, weeds, insecticide, herbicides, fungicides</p> <p>Intrinsic: allergins, lectins, phytoestrogens, glucosinolates (rape seed), saponins, tannins, ricin, sinapine, gossypol, (cotton seed cake), lipoxygenase, trypsin inhibitor, urea.</p> <p>Decomposition and rancidity test: acid value, peroxide value, etc.</p> <p>Protein quality: protein solubility or dispersibility, Nitrogen solubility, mailard reaction product, dye binding, pepsin digestibility, amino acid digestibility.</p>

- Protect the samples, the sampling instrument and the containers for samples from adventitious contamination.
- Preserve the samples in clean, dry and sterile containers. The sample containers shall be of such a size that they are almost completely filled by the sample.
- Each container shall be sealed 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. Market full details of sampling i.e. the date of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.
- Samples shall be stored in such a manner that there is no deterioration of the material.

- Sampling shall be done by in the presence of the purchaser (or his representative) and the vendor (or his representative).

Sampling procedures for feed analysis:

In all cases, at least 10% of the packages should be sampled. A minimum of approximately 1kg should be collected from each load. All cores should be combined in an airtight container. In India Bureau of Indian standards has laid down the following procedure of sampling the feeds.

Pierce (1985) recommended as follow:

Packages/bags	Number to be Sampled
1-10	1-3
11-25	2-4
26-50	3-6
51-75	6-8
76-100	8-10

All the containers in a single consignment of the material drawn from a single batch of manufacture shall constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be grouped separately and the containers in each group shall constitute a separate lot.

- Samples shall be tested for each lot for ascertaining conformity of the material to there requirements of the specification.
- The number of containers to be selected from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table

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Number of containers to be selected for sampling

Lot Size	Number of Containers to be selected
(N)	(n)
(1)	(2)
2 to 15	2
16 to 50	3
51 to 100	4
101 to 150	5
151 to 300	7
301 and above	10

- The containers 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 (seen IS 4905: 1968).

Arrange all the containers in the lot in a systematic manner and starting from any container count 1, 2, 3,.....etc, up to r and so on. Every r th container shall be with drawn from the lot to give a sample for test where $r = N/n$, r being the integral part of N/n ; where N is the total number of containers in the lot , and n the number of containers to be selected according to Table 2. If comes out to a fractional number, its value shall be taken to be as equal to its integral part.

TEST SAMPLES AND REFEREE SAMPLES

Preparation of Individual Samples

Draw with an appropriate sampling instrument equal quantities of the material from different parts of each container selected according to Table 2. The total quantity of the material drawn from each container shall be not less than 1.5 kg. Mix all the portions of the material drawn from the same container thoroughly. Take out about 0.75 kg of material and divide into three equal parts . Each portion, thus obtained shall constitute the test sample representing that particular container and shall be transferred immediately to clean and dry sample containers and sealed air-tight . These shall be labeled with particulars given under B-1.5.The individual samples obtained as above shall be formed into three sets in such a way that each set has test sample representing each container selected. One of the sets shall be for the purchaser, another for the vendor and the third for the referee.

Preparation of Composition Sample

From the mixed material from each selected container remaining after the individual samples have been taken, equal quantities of material from each container shall be taken and mixed up together so as to form a composite sample weighing not less than 0.75 kg. This composite sample shall be divided into three equal parts labeled with the particulars given under and sealed air-tight. One of these samples shall be for the purchase, another for the vendor and the third for the referee.

Referee Sample

Referee sample shall consist of a set of test samples and composite samples, and shall bear the seal of the purchaser and the vendor and shall be kept at a place agreed to between the two.

Testing of Samples

- Samples shall be tested for each lot for ascertaining the conformity of the material to the requirements of this standards.

Criteria for Conformity

A lot shall be considered as conforming to the specification when the test results on the individual samples satisfy the requirement.

Evaluation of Feed for Quality

The feeds are usually subject to following 3 types of tests :

1. Physical
2. Chemical
3. Biological

Physical Evaluation

Physical evaluation is easy but rough in nature. One must be highly trained to identify the changes in the nature of the raw material/feeds.

Colour : The appearance of the ingredient will reveal its quality. Any change in the colour of the feed ingredients gives an indication of the maturity of the grain, storage conditions, presence of toxins, contamination due to sand, possible use of insecticides/fungicides which gives dull and dusty appearance. Orange to red colour of sorghum indicates high tannin content. Browning or blackening due to heat on improper storage reduces nutritive value. Black coloured fish meal indicates the rancidity of fish oils.

Size : Size of the grains govern its energy value due to the proportional decrease/increase in seed and its coat. Smaller the grain lower will be the ME value due to more proportion of coater hulls. To evaluate the cereals weight of a fixed number of grains usually 100 grains or fixed volume is taken. Higher weight indicates a higher ME value. This technique is called Test Weight.

Homogeneity: The presence of contaminants like other grains, husks broken grains, weed seeds, infested seeds is viewed. In the oil seed cakes closer observation will reveal the presence of fibrous material, especially in de-oiled groundnut cake, the cake with hulls which contains nearly 20 to 25% crude fibre can be visually identified. Rice polish is contaminated with husk. Clumps in mineral ingredients are not suitable for premixing.

Smell:- Smell is the next best indicator just standing near the stock itself will immediately indicate any difference in the normal smell. The mill manager should familiarize himself with the normal smell of the ingredients, any change in

the normal smell of the ingredients should be viewed with suspicion. Musty Odour indicates the beginning of fungal contamination or boring insects. To detect rancidity in oil rich feed ingredients this is the best method. Odour of petroleum products is suggestive of excessive pesticide or fungicides. Leathery smell of meat meal indicates its adulteration with leather meal.

Taste:-Each ingredient has a different taste, any change in the taste like bitterness in the grains, soya, sunflower oil meal and groundnut cake indicates the presence of mycotoxins. The level of salt can be detected by tasting the ingredient and the feed. Bitter taste of rice polish indicates the rancidity of the fatty acids.

Touch :- Feeling the raw material will indicate the dryness. Chilliness indicates high moisture content. Clumps can be found out by inserting the hand inside the bag, The clumps may be due to high moisture content, improper storage, packing of fresh warm solvent extracted meal. Which crumble on application of light pressure. Clumps formed due to excess of moisture will be very hard. To evaluate rice polish, place about 25g of rice polish on the palm and close the fingers tightly and then open the fingers the rice polish will become like a solid mass if the crude fiber level is below 12% if the fiber level is high the mass will disintegrate once the fingers are opened, Further pressure will be felt when the hand is closed in high fiber rice polish.

Sound :- Dry grains on pouring down or biting will produce sound of spilling coins.

Physical Methods to Detect Adulteration or Contamination

The Common contaminant or adulterant is husk or sand. Winnowing is the best method to detect husk in the feedstuff. Sieving can be done to differentiate contaminants based on particle size. To detect for the presence of sand a weighed quantity of the grain is soaked in water then by sieving with hand the grains can be separated. The remaining water if decanted the settled sand can be weighed and the level of contamination can be assessed.

Chemical Evaluation

An analytical laboratory for the precise estimation of nutrient contents and contaminants is of utmost importance.

Analyse the feeds for proximate principles. This indicates possible constraints on usage due to the presence of excessive content of crude fibre, fat or total ash. Low CP and high CF of oil seed meals is indicative of adulteration with fibrous material. The high CF alone is indicative of adulteration with urea and or some inferior quality oil seed meals like mahua, castor or karanja cake.

The amount of acid insoluble ash is a good guide to the amount of sand or other dirt which may be present. The fish meals are usually adulterated with sand

during drying process.

It is also desirable to determine the free fatty acid content of oily materials as this will affect palatability due to rancidity of oils. The chemical composition/specifications of various animal feeds are laid down by the BIS which act as guidelines for the suppliers, buyers and the users at farm level. The protein meals should also be analysed for their amino acid contents.

Ingredient Specifications

Ingredient specifications are essential in a feed quality assurance program. Specifications serve as the basis from which purchasing agreements are written, feed/rations are formulated and ingredient inspections are performed. Ingredient description and general nutritional specifications may be found in BIS specifications for feeds and feed ingredients in India.

Specifications of the feeds must be as comprehensive as possible, realistic, must be transmitted to the seller. These are the “measuring sticks” to which the delivered material must conform. Specifications are the foundation of a quality assurance program because they serve as an understanding between nutritionist, purchasing, and production departments. A list of feed ingredients and their target nutrient level laid down by BIS are presented in Table as annexures. Some analytical procedures are given to detect the various types of adulteration.

Mahua cake : To water extract of the test feed add conc. H_2SO_4 : Violet or pink colour indicate the presence of mahua cake.

Argimona seeds: To water extract of test feed add conc. HNO_3 . Appearance of **Brown-reddish** colour indicates the presence of argimona seeds.

Detection of castor cake in feedstuffs or edible oil cakes, BIS has specified the cake methods of analysis of castor cake, linseed meal, neem seed cake, cotton seed cake

The ricin is extracted from 10 g of feed with different solvents present in castor cake only and injected to immunized rabbit and mortality is observed. The immune serum will not prevent death from any substance other than ricin. Injecting the extract of a feedstuff (containing castor cake) mixed with normal serum causes greater mortality in rabbits and rodents. This test can detect 1 ppm of ricin.

The second method of detecting castor cake is the used of potassium chlorate. When feed is treated with potassium chlorate, the castor cake is destroyed and settles down at the bottom.

Detection of Neem Seed Cake in feedstuff and edible oil cakes

The coarsely powdered feedstuff is percolated three times at room temperature with 95% alcohol. The total percolate is concentrated under reduced pressure till a thick syrupy amber coloured residue is obtained which is treated with different solvents to extract a crystalline product (N inbine). It is cautiously dissolved in concentrate sulphuric acid, the resultant brown solution changes to cherry red on addition of small quantity of concentrated nitric acid. The crystalline product gives a yellow colour with tetranitromethane. A alcoholic solution of the crystalline product shows a sky blue florescence under ultraviolet light.

Detection of Linseed meal in Animal Feeds

A little of the feed is treated with 1 or 2 drops of dilute sulfuric acid in a micro test tube. It is some times necessary to add some granulated zinc and more acid. The mouth of the test tube is covered with a disk of filter paper moisten with a drop of reagent. Depending upon the amount of hydrogen cyanide produced a more or less intense blue appears on the reagent paper. Gentle warming in water bath is advisable when small quantity of cyanide are suspected.

Detection of unextracted cotton seed cake in Animal Feeds

Weight accurately about 200 mg of sample into screw cap in test tubes and extract cycloprenoids and then measure it colormeterically.

Detection of common salt (Sodium Chloride)

Weigh 1g of sample and add 100 ml of distilled water. Stir and filter. Then the filtrate is used measure the by adding 8 ml of nitric acid solution and silver nitrate solution.

White Turbidity- indicates the presence of salt

Detection of urea

Urea is detected from the feed by addpotting the following procedure :

1. Weigh 10 of test sample and add 100 ml of distilled water. Stir and filter .
2. Pipette 2ml of standard solution and test sample into white porcelain spot plates.
3. Add 2-3 drops of cresol red indicator and add 2-3 drops of urease solution.

4. Let it stand for 3-5 minutes, if urea is present, it will form a deep red-purple spreading like a spider's web appearance, in contrast to the yellow color of the indicator.
5. Compare the test sample with the standard urea sample. This test should be read within 10-12 minutes.

Measurement of Quality of Soybean Meal :

Quality of Soybean meal is tested for the presence of two antinutritional factors trypsin inhibitors and haemagglutinins, which depress the utilization of proteins and for urease activity, an indicator of level of cooking or processing applied during the preparation of soybean meal. Both the urease enzyme and trypsin inhibitor are denatured at the same rate. Due to easier assay of urease enzyme it is accepted by the feed industry worldwide

1. Spread the sample uniformly on petri dish, glazed paper with white background. Spray cresol red and thymol blue reagents the particles.

Interpretation :

Visual Examination of Soybean Meal when Treated with Urea-phenol Red Solution*:

	Urease Activity	Approximate range of urease	Assessment
Not visible red colour	Inactive	0.00	overcooked
Few scattered red particles	Slightly active	0.05 - 0.10	properly cooked
Approximately 25% or red particles	Moderately active	0.20	properly cooked
Approximately 50% or more red particles	Very active	Above 0.20	Under cooked

*Urea - phenol red solution is made as follows. Dissolve 0.14g of phenol red in 7 ml 0.1N NaOH and 35 ml distilled water. Dissolve 21g of urea in 300 ml distilled water. Mix these two solutions together and titrate to amber colour with 0.1N H₂SO₄

Detection of Hoof or Horn In feed:

For quick test, place 2-3 particles of amber color test sample into an evaporating dish and then add 5 ml of glacial acetic acid into the evaporating dish and let it stand for 60 minutes. If hoof and horn are present, the test particles will still hard and tough. Gelatin will become soft and swollen.

Detection of Leather Meal:

Pick up brown to black test sample particles and place in petri dish and then add 3-5 drops of ammonium molybdate and let it stand for 5-10 minutes. Leather meal will give no color change. Meat and bone meal gives a greenish yellow color.

Quick Test for Quality of Fish Meal

Most water soluble NPN adulterants react with mercuric potassium iodide alkaline solution mixture and a heavy orange precipitate colour occurs. Put 2-3 g of test sample in a 100 ml beaker and add 10-15 ml distilled water and stir. After 2-3 min add 3-5 drops of test sample into white porcelain spot plates and add 2-3 drops of mercuric-potassium iodide alkaline solution mixture. A heavy orange precipitation colour indicated presence of NPN. The intensity of orange precipitation depends on the amount of non-protein nitrogen present.

Detection of Hydrolyzed Feather Meal from Fish Meal:

Hydrolyzed feather meal contains a high percentage of cystine (6-7%). When the sample is digested with sodium hydroxide, the cystine and cysteine are liberated and reaction with lead acetate gives the dark brown black color on the surface of the particle. Place one teaspoon of well mixed standard hydrolyzed feather meal and test sample of fish meal into two sets of petri dishes. Add 10-15 ml of solution A into all the two sets of each test sample. Swirl gently to spread samples evenly in each dish and let them stand for 10 min. Add 10-15 ml of solution B into each first set of petri dishes and into the second set add 10-15 ml distilled water. Mix gently by turning around each petri dish and let them stand again for 10 min. During standing, a visible browning reaction color develops until back colored particles appear in the first set of petri dish for the standard hydrolyzed feather meal. When compared to the second set (without adding solution B), no visible brown color develops after 10 mins. Compare the test fish meal sample with the standard hydrolyzed feather meal sample and also the visible browning colour between the first and second set of each test sample. If the color of these two sets differs, the fish meal is adulterated with hydrolyzed feather meal.

Decomposition Test for Animal and Marine Products:

As animal and marine products spoil, protein breaks down to amines. The residue of these bio organic amine can indicate the freshness or decomposition of the sample. If the sample is badly decomposed, the test sample will darken quickly with saturated lead acetate paper and it is not suitable for feeding.

Put 5 g of test sample into 250 ml flask. Prepare a cork, which fits tightly, with a 2" x 1/4" strip of white filter paper pinned to the bottom, moistened with saturated lead acetate. Add 50 ml dilute acid into the sample then immediately insert the cork and let it stand in a warm room for 16 hrs. If the sample is badly

decomposed, the test paper will darken quickly.

Identification of Plant Protein and Animal Protein in Feed:

Carbohydrates from plants contain starch and cellulose. When it reacts with iodine and chlor-zinc iodine solution, the starchy tissue releases a blue color and the plant fibre or cellulose develops a purple brown color when examined under a microscope.

1. Mix 1-2g test sample with 100 ml boiling water or boil the mixture for 2-3 min. Place a few ml of the cooled mixture in test tube and add 5-6 drops of iodine solution. If starch is present, the mixture turns blue.
2. Spread 1-2g test sample into a petri dish. Add 5-6 drops of chlor-zinc iodine solution and let stand for 10 min. A purple brown color indicated the presence of plant fiber, whereas yellow indicated animal fiber (protein) using a microscopic examination.

Toxins in animal feed:

The various feed ingredients should be analyzed for the toxins present in them. Which are other wise injurious to the health of animals. The examples of toxins in the various feeds are given below:

1. Gossypol in cotton seed
2. Halmagglutinins in soybean and castor beans
3. Glucosinolates in rape seed
4. Tannins in sorghum, oil seed meal, mango seed kernel, mustard oil cake and lucerne meal
5. Cyanogenic glycosides in linseed and cassava
6. Phytic acid in all cereals, oilseed meals
7. Mycotoxins, primarily aflatoxins in maize, groundnut cake, etc.

Ultra violet screening is used whereby a greenish yellow fluorescence is observed when the sample is exposed to ultra violet light to detect mycotoxins. The maximum permissible levels of aflatoxins is depleted in the Table.

One should get from the best source of supply and one should have some idea of normal levels of toxicity which may be expected.

Fish meal, meat meal and bone meal should be checked for pathogenic bacteria like Salmonella.

Maximum Permissible Levels of Aflatoxin as Stated by Different agencies

Food/Feed	Maximum level
USA	
Dairy feed, feed for immature animals	100 ppb
Feed for breeding cattle, swine or mature poultry	100 ppb
Feed for finishing swine	200 ppb
Feed for feedlot beef cattle	300 ppb
BIS	
Feeds for poultry	20 ppb
Feeds for ducks	3 ppb
ICAR, New Delhi	
Feeds for chicks	150 ppb
Feeds for broilers	400 ppb
Feeds for layers	900 ppb
Feeds for breeding stock	300 ppb

Dioxins Contamination in Animal Feed:

Dioxins and dioxin - like compounds are created by the manufacture of chlorine and such chlorinated compounds as chlorinated phenols. PCBs, phenoxy herbicides, chlorinated benzenes, chlorinated aliphatic compounds, chlorinated catalysts, and halogenated diphenyl ethers. The most toxic compound is 2, 3, 7, 8 - tetrachlorodibenzo-p-dioxin or TCDD.

Dioxins are produced as an unintentional byproduct of many industrial processes. Forest fires also release dioxins and are deposited onto the leaves of trees. Dioxins are highly toxic. Even minute amounts of dioxin cause damage to the nervous system and liver, apart from causing cancer. They can cause birth defects as well as mimic hormones that disrupt reproduction and human development. Dioxins released into the environment reach the food chain and get accumulated in fat. By far the greatest exposure to dioxin (over 90%) is from food. These include fish meal, fish oil, recovered vegetable oil, grease and many byproducts from the food industry, bleaching earths and kaolinitic clays, milk products. When these are included in animal rations dioxins get concentrated in animal products.

Since the results of a test for dioxin in animal products take several weeks to complete, more rapid testing was brought in based on the indicator substance polychlorinated biphenyl (PCB).

Microscopic Evaluation of Animal Feed:

Feed microscopy is commonly used for confirming the adulteration and identifying the adulterants (AOAC, 1970). Feed ingredients, adulterants and

contaminants must be studied under low and high magnification for distinguishing features whether coarsely or finely ground. At physical characteristics such as shape, color, and particle size, softness, hardness, and texture of the feeds are examined at low magnification of 8x to 50x. It is useful method to identify impurities/contaminants and evaluating the quality of feed ingredients. It also serves as a useful method for identifying missing ingredients in finished feed.

The plant cells and structural features of the feeds are observed at high magnification of 100x to 500x since their characters are retained after grinding or even after powdering the feed ingredients.

A feed scientist must be familiar with feed ingredients and adulterants and must have a collection of pure feed ingredients, adulterants and contaminants for the accurate and fast quality assurance results. The mashed and sieved feed should be used for a clearer observation of plant histology and microscopic appearance, heat the feed with 8% KOH steam bath for 30-45 min. If this treatment is not satisfactory, treat the fresh portion for a short time by gently heating with acidified chloral hydrate glycerol solution.

Precise characteristics on Microscopic Identification

- **Crab Products:** Orange pigmentation, segmented antennae with calcareous shell and will effervesce in diluted HCl, honey combed round cells on the outer layer of shell.
- **Fish Products:** Curved scales with concentric rays, bone exhibit lacunae with well-defined canaliculi, milky glass beads with broken surface eye lenses.
- **Shrimp Meal:** Segmented leg and antennae, thin shell with mica-like and in some areas, may appear cross-hatch type of marking, feathery delicate gill tissue, amber colored cells of compound eyes.
- **Squid Products:** Mottled body fragment with black pigment spots, tentacles or sucker pieces present, no lacunae or surface lines on internal shell fragments.
- **Blood Meal:** Spherical particle, smooth surface with glass when rubbed, dark red to almost black in color.
- **Meat Meal and Meat and Bone Meal:** Strong greasy odor, consist of hoof, horn, hair, fluff and vegetable fiber, cylindrical rods smooth muscle with alternative dark and light striated muscle.
- **Soybean Meal:** Yellow to brown oval hilum with a clear slit, pox-marked outer surface hull, hourglass and palisade cells from the hull are the major

cellular keys for soybeans and also elongated cells below the peripheral cells of the cotyledon.

- **Peanut Meal:** Thin skin with copper to red color, highly pitted cell of pod fragment and the lack of palisade cells in the testa, elongated pite in hypodermal stone cells and unique cross fiber cell of pod.
- **Sunflower Meal:** Striped or all black varieties for the hull, leathery hull with a paper-like lining. Twin hairs, united almost to their tips on the outer surface of the cypsela, unbroken pericarp fragment may appear as broken pieces in the medium, the outer epiderm of transversely elongated cells with zigzag walls.
- **Rapeseed Meal:** Many species of rape are lumped together and are difficult to identify separately by structured features. For all practical purposes, the examination of the seed coat or testa for degree of reticulation is important. The inner surface seed coat has a delicate semi-transparent, white sheet adhering to the surface.
- **Sesame Meal:** Seed coat or outer epidermal cells contain calcium oxalate crystals with black brown or yellow brown colored and granular surface.
- **Cottonseed Meal:** Long, flat and twisted fibers adhering to the hulls, kernel fragments are yellow to brown containing many round, red, brown gossypol glands. The hull edge has a light brown layer with stairstep facets. The epidermal cells are heavy walled with dark pigmented interiors. Palisade cells can also be used for identification.
- **Copra Meal:** Irregularly shaped flaky fragment with large, colorless, straight, thin walled cells of endosperm containing oil globules.

Detailed microscopic observations for fish meal and rice polish are given below:

FISH MEAL

a.	Muscle fiber:	Fiber bundles which separate under pressure, yellowish to brown colour and greasy.
b.	Scales :	Transparent, round with concentric rings, flat or curled.
c.	Sand ¹ :	Granular, crystalline or bead, like. Light brown to translucent, do not break under pressure.
d.	Urea ¹ :	Shiny, needle like crystalline appearance cracks on pressure.
e.	Meat meal ¹ :	Dark brown to black, chunky with bone pieces appearing as gray to white.
f.	Salt ¹ :	When treated with 0.1N Silver nitrate solution it turns into white precipitate.
RICE POLISH:		
a.	Polishings :	Yellowish to light brown, greasy, curly, thin and small flakes
b.	Grain pieces :	White translucent
c.	Husk ¹ :	Scaly with longitudinal Striations and yellowish.

1 Possible adulterants

3. Biological Evaluation : Biological evaluation of the feeds involve the use of animals, specialized persons to conduct the digestion and metabolism trails on the various species of livestock and poultry. These methods are time consuming.

Improvement in the quality of feed:

Improvement in the quality of feed can be done by

- 1 Choosing the best quality raw materials available
- 2 Fortifying the nutrient content of the diet with commercially available nutrients i.e. amino acids, mineral supplements, vitamins etc.
3. Using additives to enhance the availability of the nutrients e.g enzymes

The wide variations in the chemical composition of the ingredients is obtained. This is the main constraint with which the farmer and the nutritionists have to formulate the ration to maintain the quality of the feed at affordable costs. Hence choosing the best quality raw material continuously throughout the year is nearly impossible. Further , we are not in a position to reject the materials if there is variation in the specification since the availability is constant or lower and the demand is increasing .Therefore fixing the cost of the ingredient on the basis of

nutrient content and using them in the formulation with certain additives is the possible. The commonly suggested additive is the use of fiber digesting enzymes, which not only reduces the anti nutritional effects of the fiber but also enhances the digestibility of the nutrients and thereby improves the performance of the birds.

Routine assessment of the raw materials is essential. Purchase of raw materials should be based on quality and nutrient content. Formulation should be done to obtain optimal production at the lowest cost.

Suitability of stored and damaged cereals for livestock feeding:

In India the food grains produced are usually stored in bulk by the Government Agencies (FCI and CWC), and to some extent by the farmers. Food grains during storage undergo certain physical, chemical and biological changes due to the presence of enzymes and biochemicals itself and the enzymes produced by the insects pests and microbes or due to some other factors. These changes may deteriorate the quality of the grains. Usually the following changes occur in the food grains during harvesting, handling, transportation and storage:

- A. Physical changes
- B. Chemical changes
- C. Biological changes

A. Physical changes : The sound and healthy grains are shining with good luster and show hardness. The various physical changes the grains undergo during storage are, dull colour, musty odour, bores in grains, sprouting of seeds, damaged kernels due to bad weather conditions.

B. Chemical changes: Cereals are characterized by relatively low protein and high carbohydrate contents contained in kernel. The germ is rich in proteins, fats, sugars and minerals whereas the endosperm is low in protein, fat and ash contents.

The various chemical changes that occur during storage are due to increased activity of endogenous and exogenous enzymes which are responsible for quantitative and qualitative changes in carbohydrates, proteins and fats of the cereals in addition to colour, flavour and texture..

(i) Carbohydrates: In India, the temperature and relative humidity varied greatly (Temp: 6-45 C ; R.H: 22-100%) during storage which causes biochemical and physical changes in grains such as bursting and gelatinisation of starch and depending upon the moisture content. Amylases hydrolyse the starch into dextrose and maltose and significantly increase the content of reducing sugars during storage.

Storage of wheat above 12 % moisture increased sucrose, glucose,

fructose and raffinose contents. The storage of cereals at high moisture content also produces sour odour due to the production of alcohols and acetic acid.

- ii. **Proteins :** The high temperature and production of chemicals in grains during storage denature the proteins and make them less dispersable in water, deteriorates the gluten quality and increase the free amino acids contents.

The formation of certain sulphur containing amino acids impart bad odour. The free amino acids may also undergo maillard reaction combining with the reducing sugars giving browning of the grains. The type of deterioration is possible at temperature above 20⁰ C and at RH between 60-70 percent.

- iii. **Lipids:** Oxidation of lipids especially the unsaturated fatty acids results in typical rancid flavours, odour and taste. Hydrolysis of lipids also increase the fatty acid (FFA) contents which is considered as a sensitive index for the grain deterioration.

- C. **Biological changes :** Infestation of weevils, insects, microbes and sprouting affect the nutrient composition of the cereals, through various metabolic reactions occurring in the seed by the enzymes produced.

Insecticides and pesticides Residues: To control the infestation of insects ,pests and rodents in the food grains, several insecticides, pesticides and rodenticides are used. The residues of these chemicals must be within the prescribed limits as per the Prevention of Food Adulteration (PFA) Act.

Contaminants: The food grains are usually contaminated with foreign material viz stones, chaffs, poisonous weeds, excreta of insects, pests, rodents etc. which gives poor look to the grains. The limits of weed presence, uric acid and insect excreta described by the Govt. Of India (FCI) for the stored food grains.

Categorization of food grains:

On the basis of damage to the kernels, infestation of insects, pests, FCI has given the following categories of different grains.

Category	Weevilled/germ wheat	eaten/touched grains % Maize Paddy*
A	Up to 1	5
B	1-4	5-10
C	4-7	10-15
D	7-10	15-20

*Basis of categorization is same except the incorporating designation to indicate the intensity of slightly damaged/dicoloured kernels and designation are represented as 1,2,3,&4

Use of damaged food grains for feed:

As per the quality control manual of FCI the damaged food grains are classified into five categories for their disposal which may be declared fit for consumption by the livestock/poultry birds.

Sr. No.	Class	Sound/slightly damaged/touched & broken grains %	Category for which declared fit
1.	Feed-1	70-85	Poultry
2.	Feed-2	55-70	Cattle
3.	Feed-3	30-55	Industrial
4.	Manure	10-30	Manure
5.	Dumping	4-10	Dumping

Practically no information is available as far as the suitability of different grade of cereals damage storage as livestock feed is available. Before any recommendation is made in this regards, complete evaluation of these materials is required with respect to their chemicals composition., toxins, residues of insecticides, pesticides and finally the in vivo feeding value of different categories of livestock and poultry.

BIS SPECIFICATION FOR CATTLE FEED

Characteristics	Cattle (type 1)	Cattle (type 2)	Calf Starter	Calf Grower
Moisture Max%	11	11	10	10
Crude Protein Min%	22	20	23-26	22-25
Ether Extract Min%	3.0	2.5	4.0	4.0
Crude Fiber Max%	7	12	7	10
AIA Max%	3	4	2.5	3.5
Salt max% (as NaCl)	2.0	2.0	-	-
Calcium Min% (as Ca)	0.5	0.5	-	-
Available Phosphorus	0.5	0.5	-	-
Vitamin A (IU/Kg)	5000	5000	-	-

BIS SPECIFICATION FOR MINERAL MIXTURE (BIS)

Characteristics	Cattle	Sheep & goat	Poultry
Moisture Max %	05	5	03
Calcium Max%	16	30	30
Phosphorus min%	09	14	9
Magnesium Min%	04	-	0.4
Sulphur Max%	1.4	0.13	-
Salt Min%	22	-	-
Zinc Min%	0.3	0.2	0.4
Iron Min%	0.3	0.55	2000 ppm
Iodine (as KI) Min%	0.02	0.35	0.01%
Copper Min%	0.078	0.03	500 ppm
Man ganese Min%	0.1	0.08	-
Cobalt Min%	0.009	0.008	-
Flourine Max%	0.05	0.03	0.05
Total Ash%	75.0-82.0	78-85	-
AIA%	3.0	3.0	3.0
Organic Impurities	Nil	Nil	Nil

BIS SPECIFICATION FOR POULTRY FEED

Characteristics	Broiler Starter	Broiler Finisher Feed	Chick feed	Growing Chicken Feed	Laying chicken Feed	Breeder Layer Feed
Moisture Max%	11	11	11	11	11	11
Crude Protein Min%	23	20	20	16	18	18
Crude Fibre Max%	6	6	7	8	8	8
AIA Max%	3	3	4	4	4	4
Salt Max% as NaCl	0.6	0.6	0.6	0.6	0.6	0.6
Calcium Min% (as Ca)	1.2	1.2	1	1	3	3
Available P Min%	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin A (IU/kg)	6000	6000	6000	6000	8000	8000
ME Min% (Kcal/kg)	2800	2900	2600	2500	2600	2600

BIS SPECIFICATION FOR PIG AND RABBIT FEED

Characteristics	Pig feed			Rabbit feed	
	Starter	Growth	Breeding	Meat	Wool
Moisture Max%	11	11	11	11	11
Crude Protein Min%	20	18	16	16-18	15-17
Crude Fibre Max%	5	6	8	10-12	10-12
AIA Max%	4	4	4	-	-
Ether extract Min%	2.0	2.0	2.0	2-3.5	2-3.5
Vitamin A (IU/kg)	1700	1300	1300	-	-
Calcium, g/Kg	6	6	6	-	-
Phosphorus, g/Kg	6	4	5	-	-

Marking

Each container of feed should be marked or labeled giving the following information:

- a) Name and type of the material used,
- b) Indication of the source of manufacture,
- c) Batch or code number,
- d) Net mass in kg, and
- e) Date of manufacture

Classification of Mycotoxins

The classification of mycotoxins is as follows:

Mycotoxins	Fungi
Aspergillus toxins Aflatoxin B ₁ , B ₂ , G ₁ , G ₂	Aspergillus flavus, Aspergillus parasiticus <i>Aspergillus flavus</i> <i>Aspergillus ochraceus</i>
Cyclopiazonic acid Ochratoxins	<i>Aspergillus ochraceus</i>
Penicillium Toxins:	
Ochratoxins Citrinin	<i>Penicillium viridicatum</i> <i>Penicillium citirium</i>
Fusarium Toxins	
T-2 Txin, HT-2 Toxin, Diacetoxyscirpenol (DAS), Monoacetpxyscirpenol (MAS)	Fusarium tricintum <i>Fusarium solani</i>
Deoxynivaleol (DON, vomitoxin)	<i>Fusarium greminearum (Gibberella zea)</i>
Zearalenone	<i>Fusarium graminearum, Fusarium roseum</i>
Fumonisin B ₁ , B ₂	<i>Fusarium moniliforme, Fusarium proliferatum</i>
Ergot toxins	
Ergopeptines Ergovaline	<i>Claviceps purpurea</i> <i>Acremonium coenophialum</i>

Important mycotoxins in foods and feeds

The important mycotoxins in foods and feeds are as follows:

Mycotoxins	Nature of toxin
Aflatoxins* (Most ubiquitous) and Cyclopiazonic acid	(Hepatotoxins, Immunosuppression)
Ochratoxin* and Citrinin	(Nephrotoxins, Gout)
T-2 toxin* and Diacetoxyscripenol	(Mouth lesions, Loss of appetite, Skin and Gastro-intestinal irritation)
Fumonisin* and Moniliformin	(neurological disorder, Liver damage)
Vomitoxin* and Fusaric acid	(Feed refusal, Dermatotoxins)
Zearalenone*	(Estrogenic and Reproductive disorders)

*Mycotoxins to occur in feed stuffs significantly		
Important mycotoxins in forages		
Ergot alkaloids	Sporidesmin	Fescue toxin
Tremorgens	Patulin, Vomitoxin	Zearalenone

Occurrence of mycotoxins

Contamination of feedstuffs with mycotoxins is a global problem. However, in certain geographical areas, some mycotoxins are encountered more often than the others.

Environmental condition	Mycotoxins contaminating feed stuffs
Winter conditions with high moisture	Vomitoxin, Zearalenone, Ochratoxin, Diacetoxyscirpenol (DAS), T-2 toxin, Fumonisin
Warm and humid conditions	Aflatoxins, ochratoxin (produced by <i>Aspergillus</i> species only), and fumonisin

Effects on health and production performance

The physical or apparent effects of mycotoxins range from reduced feed intake and poor conversion ratio to a general inability of an animal to thrive. Symptoms vary toxin to toxin as shown below:

Aflatoxin	Damages liver and causes growth suppression.
T-2 toxin	Oral lesions in poultry
Ochratoxins	Kidney damage Poultry and pigs are prone to ochratoxin, whereas dairy animals can tolerate it even at higher levels because of its biotransformation by ruminal microbes.
Vomitoxin (feed refusal factor)	Affect mainly pigs and other animal
Zearalenone	Affects the reproductive organs in pigs, dairy cattle and poultry
Fumonisin	Cause nervous disorders in horses
Ergot alkaloids	Produce nervous system disorders and necrosis of legs and tail in livestock

It is common to observe symptoms of mycotoxin toxicity in field conditions, but an analysis of contaminated feed samples may reveal negligible levels of mycotoxins. This is usually attributed to inadequate sampling of feed, error of analysis or the presence of other unknown mycotoxins.

Mycotoxins residues in animal products - impact on human health.

The problems with mycotoxins do not, however, end in feed or in reduced animal performance, for many are actually transferred into the meat or milk. The maximum limits of aflatoxin in foods and feeds in USA and India are given in Table 1.

Mycotoxins (In decreasing order of severity) that cause Immunosuppression	Impact of mycotoxins on the immune system
1. Aflatoxin	1. Reduction in size of bursa of Fenricius and thymus
2. Vomitoxins, T-2 toxins, HT-2 toxin	2. Reducing in T-lymphocyte, B-lymphocyte and white blood cell counts.
3. Ochratoxin	3. Reduction in total serum proteins and immunoglobulin
4. Fumonisin	4. Reduction in antibody titers
	5. Reduction in serum concentration of antibiotics.

Table 1. Maximum limit of aflatoxin level in foods and feeds: U.S, India

Countries	Product	Species
United States (ppb)		
0.5 (Aflatoxin M ₁)*	Milk	Humans
20	Any food, except milk	Humans
20	Feed	All species
India (ppb)		
50	Animal feeds	Poultry and livestock

*A toxic metabolite of aflatoxin B₁, that occurs in milk

Safe level of mycotoxins in foods and feeds

Strictly speaking, there is no safe level. The risk directly depends on the level of the major mycotoxins and also on the presence and levels of other mycotoxins in feeds. What is a safe level in one farm may not be safe in another because of difference in mange mental conditions and disease prevalence. Some factors that affect the mycotoxins toxicity are: interaction of mycotoxins with pathogens, genetic variability, environmental conditions (high temperature, humidity, ammonia, etc.), sex difference and nutritional status of the poultry and livestock.

Control of mycotoxins

The present concept of mycotoxins control has come beyond the stage where the control of fungal growth is of prime concern. The current emphasis is on reducing the deleterious effects of the pre-formed mycotoxins and thereby enhancing production. Strategies to reduce the impact of mycotoxins include plant breeding for mould resistance, efficient harvesting and storage practices to minimize contamination and the development of potential commercially applicable techniques for decontaminating such commodities. Many decontamination methods have been tried that can be broadly categorized as physical, chemical or biological.

The most effective methods of neutralizing mycotoxins already in feed is by binding them to an inert compound before they can be absorbed from the intestines. The ‘ideal’ features of a good mycotoxin binder are:

- Ability to bind a wide range of mycotoxins
- Low effective inclusion rate in feed
- Rapid and uniform dispersion in the feed during mixing
- Heat stability during pelleting, extrusion, and during storage
- No affinity for vitamins, minerals or other nutrients
- High stability over a wide pH range and
- Bio-degradability after excretion

i. Nutritional modifications

Nutritional routes for protection against mycotoxins include methionine selenium and vitamin supplementation of affected diet and some plant and herbal compounds, including chlorophyll derivatives, aspartame, etc. Mycotoxins, upon being absorbed, get detoxified in liver-utilising glutathione (selenium containing compound), which is composed of cystine (derivative of methionine), thus, the metabolic level of methionine is depleted, leading to poor growth and feed efficiency.

ii. Herbal mould inhibitors

Certain herbs and herbal extracts have been found to exert inhibitory effect on mould growth and toxin production. Aqueous extracts of garlic, onion, turmeric, neem, etc., have been shown to exert anti fungal activity and / or inhibit aflatoxin production. This method, though may be beneficial to some extent, standardisation and practical implementation are not an easy task.

iii. Chemical detoxification

Among the chemicals tested for their ability to detoxify/inactivate mycotoxins ammonia, sodium bisulfite, peroxide acids, bases and gases are effective.

However, most chemical methods are not practical and they do not fulfill all the requirements, especially those concerning the safety of reaction products and the palatability of the feed.

iv. Application of mineral clays

Many chemicals have been tested for counteracting mycotoxins, and among those that are successful, very few are used commercially. These include bentonites, zeolites and aluminosilicates. Among these, aluminosilicates are found to be more effective. Hydrated sodium calcium aluminosilicate (HSCAS) at 1.0% of the feed (10 kg per ton) can significantly diminish many of the adverse effects of aflatoxin in chicken and pigs.

However, these clays have some disadvantages, like high inclusion rates and narrow range of binding efficacy. They are mainly effective against aflatoxins and appear to have little or no beneficial effect against zearalenone, ochratoxin and trichothecenes (T-2 toxin, Diacetoxyscirpenol, etc.).

v. Microbial degradation

Ruminal microbes in ruminant animals have the ability to hydrolyze ochratoxin into a non-toxic metabolite. Ensiling is a traditional technique for preserving forages by lactic fermentation. Fungi in ensiled material can produce mycotoxins under aerobic conditions. However, mycotoxins can also be degraded during ensiling.

vi. Natural and organic binder:

Esterified glucomannan has been found to bind different mycotoxins effectively. *In-vitro* Studies have revealed that esterified glucomannan can bind zearalenone up to 75%, aflatoxins up to 92% and fumonisins up to 59%. Esterified glucomannan is also shown to exhibit a moderate binding effect on T-2 toxin and ochratoxin .

Esterified glucomannan supplementation is found to be beneficial in reducing the individual and combined adverse effects of aflatoxin, Ochratoxin and T-2 toxin in broilers In a recent study, esterified glucomannan supplementation was seen improving egg production parameters, serum biochemical and hematological parameters. Broiler trials with diets contaminated by aflatoxin, Ochratoxin A and T-2 toxin have shown that dietary inclusion of esterified glucomannan improved the body weights and antibody titres significantly.

Conclusions

The most appropriate practices for mycotoxin control are:

1. Prevention of fungal growth on crops in the field, at harvest time, during storage of feedstuffs and processing of feed.
2. Not when production is at its lowest but at the time of purchase of raw materials, storage, etc., so that mycotoxin levels can be limited to a minimum
3. Good feed can become contaminated with mycotoxins in livestock and poultry sheds. This can be avoided with proper managemental practices.
4. Application of appropriate mycotoxins binder in order to achieve good productivity and economy.

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