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DEPARTMENT OF ANIMAL NUTRITION

EVALUATION OF FEEDS BY DIGESTION EXPERIMENTS
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Evaluation of feeds by digestion Experiments

Measurement of digestibility coefficients

Digestibility –is defined as that portion of feed or of any single nutrient of feed which is not recovered in faeces or in other words the portion which is acted upon by the microbes and digestive enzymes in the digestive tract and is absorbed by the system.

When the digestibility is expressed in percentage it is known as digestibility coefficient.

$$\text{Dig. Coe. of a nutrient} = \frac{\text{Amount of the nutrient in feed eaten} - \text{Amount of the nutrient in faeces} \times 100}{\text{Amount of the nutrient in feed eaten}}$$

$$\text{Apparent digestibility}(\%) = \frac{\text{Amount consumed} - \text{faecal excretion}}{\text{Amount consumed}} \times 100$$

$$\text{True digestibility}\ (\%) = \frac{\text{Amount consumed} - (\text{Total faecal excretion} - \text{metabolic losses})}{\text{Amount consumed}} \times 100$$

- The apparent digestibility of feed is less than the true digestibility.
- Digestible coefficients are estimated for all organic nutrients.
- For ash or minerals, digestibility is not determined because-

1. It does not contribute to the energy content of the feed, and
2. Most of the absorbed minerals are excreted through the gut.

The actually digested minerals can be determined by using isotopes. (labelled minerals).

Methods of Determining Digestibility-

1. In vivo method---------- A. Direct B. Indirect ---------- a).By difference

b). By indicators/ markers

2. In sacco method/semi-in vivo method

3. In vitro method

IN VIVO DETERMINATION OF DIGESTIBILITY

Digestion and Metabolism Trials-

Measurement of Digestibility by Conventional Method: Norms Adopted in Conducting Digestion and Metabolism Trials.

1. Selection of animals. M>F

2. Preliminary Period: The test feed has to be fed in constant daily amounts, as per the requirement of the animal, for an extended period. This is called as preliminary period. The purpose is to make the gastrointestinal tract free of any indigestible material coming from the feed consumed prior to the start of the digestion trial.

In monogastric animals such as pig, digestion and evacuation are usually complete in 24 hours after the test feed is ingested. In ruminants eating a roughage ration, the last residues may not be voided until 150 to 200 hrs (6-8 days) have elapsed, though 95% are usually voided within 140 hrs. So a preliminary period **of 2-3 days** (some times 3-5 days) in pigs and **7-14 days (**8-10 days) in ruminants is followed to eliminate the feed residues of previous rations from the digestive tract and to stabilize the daily feed intake and faecal output to a constant level. Water and salt licks are provided at all times.

3. Collection Period: Animals are transferred to cages or stalls and a 2-3 day adaptation period is allowed for their acclimatisation. This follows collection period **of 7-10 days** duration; A 5-7 day collection period is mostly followed.

4. Test feed and feeding management: The test feed should not be deficient in the nutrients because a deficiency of some of them may affect digestion process. The test feed should be fed at the level required for meeting the requirements of the animals.

CALCULATION OF DIGESTIBILITY COEFFICIENTS BY DIRECT METHOD

Q. A bullock was fed 10 kg hay per day and given water to drink. Over the experimental period the animal excreted on an average: 15 kg wet faeces per day. Calculate the digestibility coefficients of organic nutrients.

O.M Hay and faeces is 79.5 and 18.6. percent

Some feeds, however, can not be fed alone as they do not supply the bulk and therefore their digestibility coefficients can not be determined directly. Examples: Concentrates (oilseed cakes, cereal grains, concentrate mixture). Similarly, non-maintenance type of roughages like straw, stovers, dried grasses, etc. do not supply the required amount of nutrients and so they can not be fed alone to the experimental animals. In aforementioned cases digestibility is determined by indirect methods.

Indirect Method of Determining the Digestibility: Digestibility by Difference

Here two or more digestion trials are conducted. In the first trial, a basal maintenance type fodder is fed and the digestibility of nutrients is determined. In the second trial basal maintenance type fodder and the test feed (concentrate feed e.g. oilseed cake) are fed together. Digestibility of nutrients of the test feed (oilseed cake) is calculated by difference on the

assumption that the nutrients in the original basal roughage have the same digestibility. In case of determining the digestibility coefficients of poor quality roughage (non-maintenance type), three digestion trials are conducted. In the third trial the test forage (non-maintenance type) is fed along with a concentrate feed (oilseed cake) and the digestibility of nutrients of the test forage is determined.

Calculation of Digestibility Coefficients by Indirect Method

Example

A cow was fed 4.5 kg of hay and 1.4 kg of cottonseed daily. The daily average output of the faeces on the combined ration was 10 kg. Calculate the digestibility coefficients of the cottonseed. (Digestibility of grass hay was determined in the first digestion trial. Hay and cottonseed were fed in the 2nd digestion trial. Digestibility of cottonseed has to be calculated by indirect method with the help of digestibility by difference.

From the 1st trial data, digestibility of cp of hay was 43 %

CP of hay, cotton seed and faeces are 4.8%, 18.2% and 2%.

Associative Effect of feed:-

Calculation of digestibility coefficients by the difference method may not be very correct since the addition of oilseed cake to the basal diet may influence the digestibility of the basal diet. But the credit is given to the supplement i.e. oilseed cake assuming that the digestibility would remain the same when the basal diet was fed alone. The effect of supplement e.g., oilseed cake on the digestibility of the basal roughage is known as associative effect of feeds. This method has a major flaw because feeds interact with each other, especially during fermentation in the rumen. This effect, associative digestibility, is caused by the extent or rate of digestion of one ingredient being either enhanced or inhibited by the presence of constituents in the other feed. For example, groundnut meal, which is rich in ruminally available nitrogen, will improve the digestion of a low-quality, low nitrogen forage such as rice straw.

Digestibility Determination in Poultry

Determination of digestibility of organic nutrients in poultry is little complicated since the nutrients are excreted from a single opening, the cloaca. There are two ways by which digestibility, of nutrients can be determined in poultry. One is the surgical means where faeces and urine are voided separately. The second method is by chemical means through which urine nitrogen and faeces nitrogen can be separated. In the urine the nitrogenous compound is uric acid and ammonia whereas in faeces mostly protein (true protein) is excreted.

Indicator Method of Determining Digestibility

The conduct of a digestion trial is obviously a laborious and time-consuming procedure. There is an indirect method using 'inert reference substance' as an indicator/marker. This method is useful when we cannot make total faecal collections.

The ideal specifications of an indicator/marker are

1. It should be totally indigestible and unabsorbable.

2. It should not have any pharmacological action on the digestive tract. It should be inert to the digestive system.

3. It must mix intimately with and remain uniformly distributed in the digesta.

4. It should pass through the tract at a uniform rate and should be voided entirely.

5. It can readily be determined chemically, and

6. Preferably be a natural constituent of the feed under test.

Indicator/marker-

1. Internal or natural indicator i.e. component of a feed. e.g. **Lignin, Silica, Acid Insoluble Ash, n-alkanes.**

2. External marker e.g. chromic sesquioxide (chrome green or **chromic oxide**, Cr_2 O3), magnesium ferrite, **carmine red**, chromium mordanted plant fibre, lanthanum and ytterbium chloride

Radioactive isotopes- ^{51}Cr-EDTA and ^{144} Ce; rare earth elements-cerium (Ce), Ytterbium (Yb), Samarium (Sm);

Chromic oxide is the most commonly used marker for digestion trials **with avians, swine and carnivorous species.** In herbivorous animals, it may not yield accurate result. Nowadays it is common to find the term 'marker' more in use rather than 'indicator'.

The digestibility of a nutrient is calculated by estimating the concentration of the indicator/marker in feed and faeces and that of the nutrient in the feed and faeces without the quantitative collection of total faeces and measuring the feed consumption.

$$\textbf{Dig. coe. of a nutrient} = 100-\left[100\times \frac{\%indicator\ in\ feed}{\%\ indicator\ in\ faeces} \times \frac{\%Nutrient\ in\ faeces}{\%\ Nutrient\ in\ feed}\right]$$

The assumption here is that the reference material is excreted uniformly and therefore a small amount of faeces collected at any time during 24 hrs should be sufficient to provide the amount of nutrient per unit of indicator (reference material). But in most of the external markers diurnal variation has been reported and chromic oxide, for example, was recovered only 94%. Hence the formula has been changed (Lucas, 1952) to

$$\textbf{Dig. coe. of a nutrient} = 100-\left[100\times \frac{\%recovery\ of\ indicator\times\%indicator\ in\ feed}{\%\ indicator\ in\ faeces} \times \frac{\%Nutrient\ in\ faeces}{\%\ Nutrient\ in\ feed}\right]$$

This method has been applied successfully in horses, swine, chickens, rabbits, foxes, minks, men, etc., in addition to ruminants (Schneider and Flatt, 1975).

Administration of Chromic Oxide

The required dose of chromic oxide (10 g) is impregnated on cellulose or paper, and enclosed it in a gelatin capsule and administered to the experimental animals. Chromic oxide was estimated in the diet and faeces samples.

Uses of Markers-

1. Measurement of digestibility coefficients without total faecal collection

2. Measurement of herbage intake in grazing animals

3. Markers are also used for quantifying rate of passage and extent of digestion in different segments of the gut. Rare earths (lanthanam, samarium, cerium, ytterbium and dysprosium) may be used as reliable markers of particulate phase of digesta. Polyethylene glycol (PEG), chromium EDTA and cobalt EDTA are liquid phase markers in ruminant studies.

Laboratory Method of Determining Digestibility

In vivo determination of digestibility in the animal requires a minimum of four animals and a large quantity of the feed sample, and daily record for intake of feed and outgo of faeces are to be determined. This requires metabolic cages or metabolism stalls and other accessories. This procedure is not only costly but also very laborious. Various techniques have been used to determine the digestibility of feeds without feeding them to the animals.

1. Digestibility of feeds and total digestible nutrients (TDN) can be calculated from chemical composition with the help of regression equations

2. Semi in vivo technique In Sacco method

3. In vitro technique

Semi - in vivo Technique

Digestibility/Degradability of feeds in the rumen can be determined by keeping the feed sample in bags which are immersed in rumen contents of rumen fistulated animals. The bag is made of nylon, dacron or silk cloth which is indigestible (Natural fibre is made of cellulose and is digested by cellulolytic bacteria in the rumen) and should be of very fine mesh so that the test feed particles should not pass out of the bag undegraded but at the same time it should allow the rumen microbes to enter into the bag and act on the test feed. The bags, on removal at different time intervals are washed till the wash water is clear and dried at 60°C for 48 hrs. The per cent disappearance of dry matter, nitrogen/crude protein, different fibre fractions, etc. are determined. This technique is called in sacco or in situ or semi in vivo technique.

Applications of the Technique

1. This technique provides a powerful tool for initial evaluation of feedstuffs, and is useful in screening, rapidly, large number of samples developed in forage breeding experiments.

2. This technique is helpful to understand the rumen processes. It is possible to vary the factors within the bag or within the rumen.

3. Degradation of protein supplements: A test protein supplement was incubated in the rumen.

Limitations-

The technique has certain inherent limitations. The test feed in the bag is not subjected to the total ruminal experience, i.e., mastication, rumination and passage. What is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds.

Factors that Affect the Degradability

The factors known to affect the degradability results obtained with the in sacco technique are particle size of the test feed, bag porosity, sample size to bag surface ratio, diet of the animal, bags per animal, animal species, number of animals, number of replicate bags per animal per incubation time, positioning of bags in the rumen, incubation length, timing of bag introduction in the rumen and pre ruminal soaking. Particle size of test feed, bag porosity and sample size to bag surface ratio are important parameters for among the laboratory comparisons of the degradability results of a feedstuff.

In Vitro Digestibility Technique

Digestibility of feeds can be estimated in the laboratory by using in vitro rumen fermentation methods. This method is used to rapidly screen large number of forage samples in plant breeding experiments for ranking them in the initial stages of developing quality fodder. This is also useful in evaluation of newer feedstuffs such as unconventional feeds.

The in vitro techniques developed and modified so far could be discussed under two main heads.

a. One-stage technique: The principle involved is that rumen microbial digestion of animal is simulated in laboratory where the feedstuff is incubated with rumen liquor at 39°C under anaerobic conditions in an artificial rumen. This technique involves the test feed sample, artificial saliva and the rumen inoculum. This mixture is placed under anaerobic conditions and incubated at 39°C a specified period. After incubation, the samples are removed and **the disappearance of dry matter (IVDMD) or organic matter (IVOMD) i**s determined and expressed on per cent basis.

b. Two-stage technique: First stage simulates the digestive process in the rumen. The residue left after the first stage is further treated with acid- pepsin solution (Tilley and Terry 1963) or with neutral detergent solution (Van Soest et al., 1966).

The acid-pepsin digestion (2nd stage) stage simulates the in vivo breakdown of feed and microbial protein by the digestive enzymes of the lower gut. The 2nd stage procedure with neutral detergent solution estimates the true digestibility rather than the apparent digestibility of test forage sample because neutral detergent solubilises bacterial cell wall and other endogenous products.

VIVAR Technique: An **in vivo artificial rumen (VIVAR)** was developed for studying nutrient utilization by rumen microorganisms under controlled conditions of rumen. The VIVAR tube is made up of stainless steel or glass and fitted with the semipermeable membranes. The rumen microflora pass through the semipermeable membrane and degrade feed sample present inside the VIVAR tube, but the sample particles can not move outside. After completion of the fermentation period, VIVAR tube is removed. The DM disappearance may be recorded by difference in weight of the sample and the residue left in VIVAR tube.

Rusitec: The continuous culture fermenter, such as "**Rusitec"** has become popular. It can be a partial substitute for in vivo animal experimentation. This 'rumen simulation technique' was first designed and developed in 1977 by In Vitro Digestibility Technique.

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Factors Affecting the Digestibility of Feedstuffs

A large number of factors affect the digestibility of nutrients. These can be broadly grouped into as follows.

A. Animal factors

B. Plant factors

C. Feed preparation.

A. Animal factor-

a. Species of the animal:

b. Age of the animals.

c. Work: Light exercise/work seems to improve digestibility of feeds while heavy exercise/work depresses it.

d. Individuality: Individual variation of as much as 25% has been observed in the digestive ability of the same feed among animals. However, most animals have shown variation of about 4-5%

e. Level of feeding: Generally, higher level of feeding results in faster rate of passage of the digesta through the alimentary canal. At higher level of feed intake the digestibility of DM and of various nutrients falls due to less retention time in the alimentary canal. Such effect is more significant in the case of ruminants, but has been observed in swine as well.

B. **Plant factors**-Chemical composition of feed: Generally grains are well utilized by all class of livestock. The digestibility of the forage is closely related to its chemical composition. The chemical composition of the forage is affected by a number of factors like soil composition, manuring and fertilization, water supply, stage of maturity of the plant, frequency of cutting, variety and strain of the plant, climate, etc., the predominant factor being the stage of maturity when cut. Differences among varieties within the same species may be due to the physical composition of the plant i.e, leaf to stem ratio, soil fertility, etc. Early-cut fodder has higher digestibility than late-cut. The protein, minerals and vitamins decrease while crude fibre increase as the plant matures.

C. Preparation of Feed

a. Particle size of the feed. If particle size is less than 600 µ, pigs may suffer from gastric ulcers. If roughages are ground to fine grinding, digestibility of fibre is decreased while total consumption is increased due to increased rate of passage. Rumen fermentation pattern is also changed due to fine grinding of feed.

b. Soaking of grains and feed in water before feeding generally increases digestibility.

c. Processing of grains/feed: Processing is done to increase palatability, digestibility and thereby feed intake. Boiling, steam processing, micronization, pelleting, extrusion cooking improve their digestibility. However some processing methods depress digestibility due to increased DM consumption and the eventual faster rate of passage. This is more conspicuous in pelleting of roughages where digestibility of DM and crude fibre decreases. Digestibility of nutrients falls due to less retention time in the gastrointestinal tract.

d. Nutrient content in the ration/Ration composition:

(i) Protein level: When several feeds are fed in a ration, one feed may influence the digestibility of the other. This 'associative effect' of feeds on one another's digestibility is more evident in the case of ruminants when the addition of a protein or NPN compound to a low protein ration increases the microbial digestion of the crude fibre by stimulating the growth of microorganisms in the rumen. Thus, as the dietary protein level increases, the digestibility of all the nutrients increase. Similarly, as the dietary protein level is lowered, the digestibility of all the nutrients decrease.

(ii) Carbohydrates: The nature and level of dietary carbohydrates affect the digestibility of all nutrients present in the diet. In ruminants, **excessive levels of soluble carbohydrates (e.g. molasses 7% and above) result in lower microbial breakdown of crude fibre**. It tends to depress not only the digestibility of cellulose, hemicellulose, etc. but of the other nutrients also. High crude fibre content of mixed diets decreases their digestibility. The higher the percentage of crude fibre in a ration, the lower is the digestibility of DM and all other nutrients.

(iii) Lipids: Addition of oil or fat in a diet increases the digestibility coefficient of ether extract, as such fats have higher digestibility than other constituents of the ether extract. **Higher levels of fat in the diets generally reduce the digestibility of other nutrients, particularly of dietary fibre.**

(iv) Minerals: In the diets of pigs and poultry, mineral content does not seem to influence the digestibility of other dietary constituents while mineral deficiency produces more severe deficiency symptoms in their body. Deficiency of minerals in herbivorous animals limits the growth of microorganisms and this will reduce the digestibility of crude fibre and of other nutrients as well. Adequate amount of salt and water tend to improve digestibility.