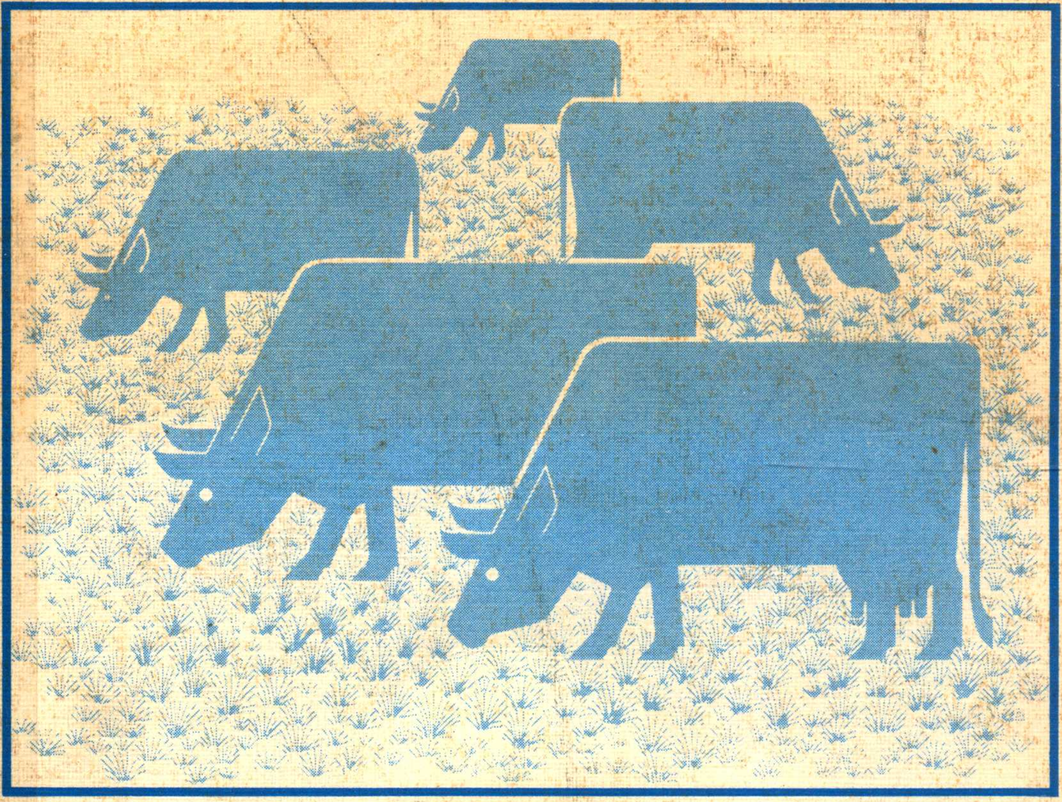

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Laboratory Training Manual on the Use of Nuclear Techniques in Animal Nutrition

JOINT FAO/IAEA DIVISION
OF ISOTOPE AND RADIATION APPLICATIONS OF ATOMIC ENERGY
FOR FOOD AND AGRICULTURAL DEVELOPMENT

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1985

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LABORATORY TRAINING MANUAL ON THE USE OF NUCLEAR TECHNIQUES IN ANIMAL NUTRITION

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FOREWORD

For many years the Animal Production and Health Section of the Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development has encouraged research both into the causes of the current low levels of animal productivity that exist in many countries and into the search for solutions. As part of these efforts, the Division has given and continues to give high priority to work aimed at defining the nutritional requirements of livestock and at evaluating and improving pastures and other locally available potential sources of feed. The benefits to be sought and achieved through such support are increases in animal productivity (e.g. growth rates, reproductive performance and milk production) brought about by increasing feed intake and digestibility or both, as well as by increasing the efficiency with which the raw materials are utilized.

The planning and conduct of useful research and the ability to interpret and make use of the data that derive from it require, amongst other things, an ability to handle techniques. This can only come from training. Since its foundation some 20 years ago the Joint FAO/IAEA Division has placed considerable emphasis on the training of scientists from developing Member States through the organization of Training Courses and individual Training Fellowships, and through the production of Training Manuals which provide information that rarely appears in scientific papers. Such Manuals do not assume extensive knowledge of the subject covered and they include practical exercises which are described in sufficient detail to enable them to be performed with minimal supervision.

This particular Manual has been produced along similar lines to those on animal parasitology (Technical Reports Series No.219) and reproduction (Technical Reports Series No.233), although substantial changes have been made to the section on basic nuclear theory and to the exercises on radioactivity counting. The present Manual includes a detailed consideration of tracer methodology, and exercises in which isotopes are used to measure a wide variety of parameters relating either to the nutritional status of the animal as a whole, or to particular organs or systems having a direct bearing on feed digestion and utilization.

It is important, however, to stress that this Manual includes descriptions of methods that do not directly involve radioactive or stable isotopes since it has to be recognized that while isotopic tracer methods provide a powerful tool for the research nutritionist, they cannot and should not be used in isolation.

Indeed, the types of question that can be answered most effectively by isotopic methods will generally have arisen from feeding trials, and will not have been resolved by further such trials or other simpler procedures. Isotopic methods should therefore be aimed at the understanding of basic principles in order to rationalize the more applied research.

The IAEA and FAO would like to thank the scientists responsible for preparing this Manual: Dr. J.W. Czerkawski of the Hannah Research Institute, Ayr, UK, who spent much of his time working on the Manual during the tenure of a one-year sabbatical leave position with the Animal Production and Health Section of the Joint FAO/IAEA Division; Dr. D. Beever of the Grassland Research Institute, Hurley, UK; Dr. M.C.N. Jayasuriya of the Joint FAO/IAEA Division, Vienna; Dr. F.W. Lengemann of Cornell University, Ithaca, USA; Dr. R.C. Noble of the Hannah Research Institute, Ayr, UK; Drs. E.R. Orskov and P.J. Reeds of the Rowett Research Institute, Aberdeen, UK; Dr. B.F. Sansom of the ARC Institute for Animal Diseases, Compton, UK; and Dr. B.A. Young of the University of Alberta, Edmonton, Canada.

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SOME BASIC SYMBOLS AND UNITS FREQUENTLY USED IN THIS MANUAL

Symbol	Description	Dimensions and/or units
Z	atomic number, i.e. proton number	
A	mass number	
A_r	relative atomic mass	unified atomic mass units (u)
M, M_{Ca}, M_{CaSO_4}	gram-atomic or gram-molecular mass	grams (g)
N_A	Avogadro's constant (number)	$N_A = 6.022 \times 10^{23}\ mol^{-1}$
$T_{\frac{1}{2}}$	radioactive half-life	time, e.g. years (a), days (d), hours (h), minutes (min), seconds (s)
λ	radioactive decay constant	inverse time, i.e. s^{-1}
t	time in general	time, i.e. s
T	counting time (duration of)	time, i.e. s
C	accumulated counts in time T	counts
R (= C/T)	count-rate including background or blank	counts per second (counts/s)
R_b	count-rate of background or blank	counts per second (counts/s)
R_s	count-rate of sample	counts per second (counts/s)
ϵ	counting efficiency = counting yield	counts per 100 distintegrations
A*	activity	becquerels (Bq), curies (Ci) (becquerels ≡ disintegrations per second)
A	specific activity of a radioisotope *or* atoms % excess of stable isotope	activity per gram or mole (e.g. kBq/g, TBq/mol, mCi/g, etc.)
S	amount of material (substance being traced)	mass (i.e. grams) or moles
$T_{\frac{1}{2}, biol}$	biological half-life (physiological elimination)	time, e.g. s
$T_{\frac{1}{2}, eff}$	effective half-life (including effects of physiological elimination and radioactive decay)	time, e.g. s

Symbol	Description
LH	luteinizing hormone
FSH	follicle-stimulating hormone
PRL	prolactin
GnRH	gonadotrophin-releasing hormones
PMSG	pregnant mare serum gonadotrophin
TSH	thyroid-stimulating hormone
TRF	thyroid-stimulating hormone release hormone
CBG	corticosteroid binding globulin
SHBG	sex hormone binding globulin
NSB	non-specific binding or B_{bl}
MB	maximum binding or B_0
B_0	maximum binding or MB
PL	placental lactogen
g	relative centrifugal force
EDTA	ethylene diamine tetracetic acid, disodium salt
PEG	polyethylene glycol
M	molar solution, containing the gram molecular weight of the solute dissolved in 1 litre of solvent

PART I. POTENTIAL OF ISOTOPIC TECHNIQUES IN ANIMAL NUTRITION

I–1. NUCLEAR TECHNIQUES IN AGRICULTURAL RESEARCH

Nuclear techniques find widespread application in agricultural research and these applications can be divided into two main groups. In one group nuclear energy is used to modify some of the properties of substances; this includes such processes as irradiation of food to improve its storage properties or, in the case of lignocellulose feeds, to make them more digestible. Within this group one could also include the irradiation of parasites in the production of vaccines and the sterilization of insects in various pest control schemes. In the other group, on the other hand, the objective is to label substances without modifying their physical and chemical properties, so that they can be used as tracers and yet behave normally. Radioisotopes are the best tracers, since by virtue of their radioactivity they can be detected and measured in extremely low amounts and since their behaviour in chemical reactions does not differ from that of the corresponding stable isotopes.

Biochemistry is the basis of most life sciences, including Animal Nutrition. It is not an exaggeration to say that much of the remarkable progress that has been made in biochemistry during the last 20–30 years would have been impossible without the use of tracer compounds and particularly isotopic tracers. The availability of radioisotopes makes it possible to measure the transfer of substances within intact, complex biological systems, such as animals or plants. Moreover, by labelling specific parts of complex molecules with radioactive atoms, it is possible to determine the fate of that part of the molecule – a technique of considerable value in animal nutrition where the main interest is in the fate of nutrients in the digestive tract, and the absorption and utilization of these substances by the animal. This is even more important in the nutrition of domestic animals (predominantly ruminants in developing countries), where nutrient conversions are subject to intervention by a complex microbial population in the digestive tract. In these circumstances, the nutrients that are actually used by the host animal are often quite different from those that are eaten. Animal productivity of animals could easily be measured without isotopic tracers, by comparing inputs and outputs, but it would be very difficult to find out what is going on inside the system and how to make it more efficient. At the molecular level isotopic methods can tell us which particular metabolic pathway operates within a system. Often it is possible to alter these pathways by changing the conditions and thereby make the system more efficient.

Isotopic techniques are also often necessary to answer questions of a more practical nature, e.g. for the measurement of the rate of production of volatile fatty acids and the rates of synthesis of microbial matter in the rumen, which are the major sources of energy and protein for the host animal.

Techniques should not be used for their own sake; they should be used to answer questions and to solve problems. Undoubtedly, the use of nuclear tracer techniques can answer many questions and has contributed to the solution of many problems. The major objective of this book is to make the use of these techniques easier and more successful in developing countries, where animal nutrition is such an important determinant of animal productivity.

I–2. OBJECTIVES OF RESEARCH ON ANIMAL NUTRITION

The primary objective of research on animal nutrition is to find methods of feeding animals that are either more efficient or cheaper in converting elements of the animal's diet into products that are useful to man. The most important products are foods of high value, such as milk and meat, but other products or uses – for example, wool, hair, furs, leather, fertilizers, draft power – are of economic importance. This primary objective is the same for both the developed and the developing countries of the world. However, the priorities for research in the two groups of countries are different because of differences between them in climate, crops and economic structure.

In the developed countries there is generally no shortage of feedstuffs of adequate quality and in the event of a shortage, for example of protein, the deficit can be made up by imports from other countries. In contrast, in many developing countries there are serious shortages of conventional feedstuffs, supplies of which often have to be supplemented with byproducts of other indigenous crops. It is too expensive for these countries to import feedstuffs from abroad. As a result of these differences, nutritional research in the developed countries is now concerned mainly with improving the efficiency of already productive systems, whereas in the developing countries research is necessary simply to raise the productivity of animals and to obtain improved utilization of locally available feedstuffs and resources.

I–3. RESEARCH NEEDS (OR QUESTIONS TO BE ANSWERED)

In order to assess the extent to which the nutrition of an animal is adequate, it is necessary to know the answers to two basic questions. First, what are the animal's total present requirements in terms of energy, protein, fat, minerals, etc. for maintenance and for growth, pregnancy and lactation and, secondly, to what extent does the animal's diet satisfy these requirements? These questions can be answered by analysing, first, the costs in terms of energy, protein, etc., of maintaining the animal at a stable weight, or producing a litre of milk or a kilogram of meat, etc., and, secondly, by analysing the proportions of the total dietary contents of energy, protein, etc., that are utilized for these purposes, i.e. the efficiency of feed utilization.

Unfortunately, although the answers to these questions are often already available, particularly in the developed countries, they do not on their own help to decide whether a particular animal production system is efficient or not. Thus a balance can be achieved between the requirements of an animal and the efficiency with which it utilizes its feed at any level of productivity, either low productivity balanced by a low feed intake and/or feed utilization, or high productivity balanced by high feed intake and high efficiency of feed utilization. To assess the possibilities for improvements in productivity, the answers to two further questions are therefore also needed. First, is the animal achieving its genetic potential or optimal rate of production; secondly, is the quantity or quality of the diet being fed limiting the animal's capacity to achieve this potential? If it can be demonstrated that the diet is a limiting factor, then animal nutrition research must tackle a final question: is it possible to get closer to the animal's optimal productivity by making modifications to its diet?

How can these questions be answered and what role do radioisotopes have in research designed to answer them?

I–4. METHODS FOR PROVIDING THE ANSWERS

Nutritional research on animals began long before radionuclides became available and much valuable information can still be obtained by using the classical methods. It is important to understand this fact and not to be misled by the power and elegance of radiotracer methods into believing that they are necessarily the first line of attack in solving practical problems. Very often much simpler methods can give basic information which can be used to effect improvements in animal nutrition. Radiotracers come into their own when it is necessary or desirable to measure, for example, the rates of particular metabolic processes, or the rates of flow of different types of digesta through the gastro-intestinal tract or to know whether a gain in weight has been the result of an increase in lean meat, fat or water or to know the partition of the various constituents of the diet into these components. Radiotracers can increase our fundamental understanding of the processes involved in animal nutrition, but these increases in understanding do not necessarily lead to immediate improvements in methods for overcoming practical problems. There is still a need for simple empirical or observational methods.

I–4.1. Non-nuclear methods

It is essential to analyse the animals' feedstuffs in terms of their energy density and content of oil, fibre, digestible organic matter, carbohydrate, protein (both rumen degradable and rumen undegradable), minerals and vitamins. Radiotracer methods provide only little help with these analyses.

The response of the animals to different diets, whether conventional or unconventional can be measured by traditional balance methods. Even when it is impossible to measure changes in weight, for lack of a weigh-bridge, it is possible to estimate changes in the condition of animals by simple methods such as measuring their height and girth at the withers or their condition score. Much information can be gained by such simple, cheap methods.

I–4.2. Nuclear methods

Some nuclear methods can measure more accurately what can already be measured by non-nuclear methods. For example, the size of some body compartments (e.g. rumen or plasma volume) can be measured by dilution techniques using either non-nuclear or nuclear tracers (see Exercise 30). Similarly, absorption can be determined by means of either a radioactive or a stable non-absorbable marker (see Exercise 42). If the nuclear technology is available, these quantities can be measured more accurately and more easily than by the conventional methods, but it would be unwise to invest in nuclear technology when conventional non-nuclear methods can provide adequate information. However, nuclear methods can provide information that is unobtainable by non-nuclear methods. For example, total body water can be measured easily with a single small dose of tritiated water. This measurement gives valuable information about body composition and, if doses of ^3H_2O are administered at intervals, about changes in body composition with growth which can be obtained in no other way. Similarly, many aspects of carbohydrate, fat, protein and mineral metabolism can be only investigated with radiotracers. These aspects are fundamental to an understanding of how the nutrients eaten by animals are converted into the products that are useful to man. Careful application of nuclear techniques to animal nutrition research can be of great assistance in improving food supplies in the developing countries.

I–5. THIS MANUAL

This Manual is planned to introduce the basic principles of work with radiotracers in a logical order. Each principle is then illustrated by means of practical exercises, which are described in detail so that students can perform them with little tutorial supervision.