## ANALYTICAL PROCEDURES IN ANIMAL NUTRITION AND FEED TECHNOLOGY



There are number of methods for each measurement reported in literature and it poses some difficulty in selecting a suitable method particularly for those who have newly entered the research field. Compilation of appropriate methods for a wide range of measurements employed in biological research is often helpful to researchers and technicians and the present book is an attempt in that direction. The book entitled "Analytical Procedures in Animal Nutrition and Feed Technology" authored by "Vinod Kumar, Muneendra Kumar and Raju Kushwaha" has been designed to meet the needs of the research students, teachers, scientist of department of animal nutrition and personnel of feed industry involved in feed analysis and quality control. The main purpose of this compilation is to make available a wide range of analytical and biochemical techniques evolved out of actual experience of research workers. Content of this book will be helpful in understanding the general principles and methodology behind preparation of standard solution, preparation, processing and preservation of samples, analysis of proximate principles, fibre fraction determination, mineral and vitamin estimation, fatty acid and amino acid analysis, estimation of energy content, in vivo, in sacco and in vitro methods of feed evaluation, rumen liquor analysis and tests for evaluation of quality of silage. This book is designed to give rapid and easy access to the recent test and techniques used in evaluation of feedstuffs, enteric methane measurement, rumen study, detection of adulteration in feed, determination of harmful constituents and pesticide residues in feeds and fodders. The text will also helpful in better understanding about recent techniques like atomic absorption and inductively coupled plasma spectrophotometer, chromatography, near-infrared-spectroscopy, calorimetry, sulphur hexafluoride tracer technique etc. Each section of book provides a structured approach to learning by covering the topics in a uniform and systematic format.

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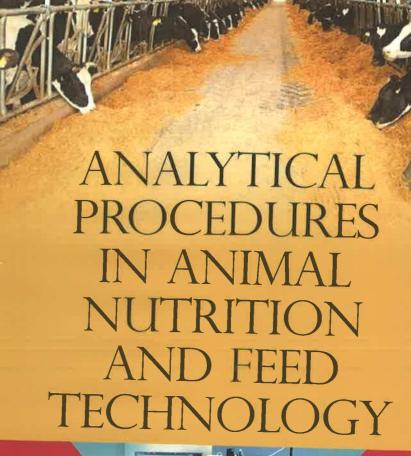


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## Analytical Procedures in Animal Nutrition and Feed Technology

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Date: October 31, 2017

#### **FOREWORD**

I am very glad to know that Drs. Vinod Kumar, Muneendra Kumar and Raju Kushwaha have authored the book titled "Analytical Procedures in Animal Nutrition and Feed Technology" keeping in view the requirements of students, scientists, teachers and others involved in the field of animal nutrition. This book will be very useful in understanding the basic principles and methodologies involved in preparation of different standard solutions, proximate analysis, fiber-fraction determination, estimation of minerals, vitamins, fatty acids and amino acids etc.

This book has been designed to give rapid and easy access to the recent and well-validated tests and techniques used in evaluation of feedstuffs, rumen fermentation studies including methane estimation, detection of adulterants, harmful constituents, and determination of pesticides residues in feedstuffs and animal products.

I am sure this book will enhance the practical knowledge and analytical skills of students, scientists and technicians involved in academics especially those who are involved in quality control in the feed-industry.

I heartily congratulate all the authors of this book and wish all success in their future endeavors and academic pursuits.



(Satish K. Garg)

## Preface

Every sector of the livestock industry, the associated services and the wellbeing of both animals and humans are influenced by animal feeding. Proper animal feeding is the supply of a diet balanced in all nutrients and free from deleterious components. The availability of accurate, reliable and reproducible analytical data regarding nutrients composition is imperative for proper feed formulation. Also only reliable data can lead to the generation of sound scientific data. Therefore, better understanding of analytical procedures and laboratory techniques used in animal nutrition is required. The text in book represents an attempt to summarize and consolidate a considerable amount of information relative to principles, methodology and experimental techniques that are commonly used in animal nutrition studies. Basically, the text is an outline of the author's laboratory manuals with attempts made to expand and reference where possible. This book is designed to give rapid and easy access to the recent test and techniques used. The content can be divided into various sections including preparation of standard solution, proximate analysis, mineral and vitamin estimation, analysis of energy content, fatty acid and amino acid, enteric methane measurement, rumen liquor analysis, determination of quality of silage, detection of adulterants and harmful constituents in animal feedstuffs. The authors hopes that this book will also serve as useful reference to understand recent techniques like atomic absorption spectrometry, inductively coupled plasma-atomic emission spectrophotometer, gas and liquid chromatography, thin layer chromatography, high performance liquid chromatography and near-infrared-spectroscopy etc. Each section of book provides a structured approach to learning by covering the topics in a uniform and systematic format. The methods and techniques are detailed in a straight forward and lucid manner. This book will be useful for research students, teachers, scientists and laboratory analysts involved in feed analysis and quality control.

Our thanks to Dr. Debashis Roy, Dr. Shalini Vaswani and Dr. Avinash Kumar for the important contribution made in the preparation of this book. I appreciate the contribution of staff of animal nutrition department for providing us conducive environment for designing of this superior manuscript.

## List of Tables\_\_\_\_

1.1	commonly used chemicals5
1.2	Common examples of acid-base indicators5
1.3	Amount of acid/alkali required for preparation of standard solution9
2.1	Amount of biological sample taken for bio-chemical analysis
5.1	Classification of minerals
5.2	Elements detectable by atomic absorption spectrophotometer
6.1	Wavelengths used in the application of NIR-spectroscopy55
6.2	NIR-wavelengths and their association with chemical structures
6.3	Error in NIR-spectroscopy
12.1	Composition of different solutions
12.2	Composition of rumen inoculum
13.1	Physical characteristics of rumen fluid100
13.2	Chemical characteristics of rumen fluid101
13.3	Microscopic examination of rumen protozoa102
15.1	Confirmation of feeds by physical evaluation120
15.2	Precise characteristics on microscopic identification122
15.3	Visual examination of soybean meal when treated with urea-phenol red solution
15.4	Visual examination of presence of heavy metals in animal feed
16.1	Common anti-nutritional factors present in animal feeds and fodders132

## List of Figures\_

7.1	Diagram of bomb calorimeter	60
12.1	Components of permeation device	91
12.2	PVC collector canister (yoke) parts	91

## Contents \_\_\_\_\_

Abo	ut the Authors
Fore	wordvi
	aceix
List	of Tablesx
List	of Figuresxii
1.	Preparation and Expression of Standard Solution1
2.	Preparation and Processing of Sample11
3.	Proximate Analysis21
4.	Estimation of Fibre Fraction
5.	Determination of Mineral Content39
6.	Determination of Chemical Composition by Near-Infrared Spectroscopy
7.	Determination of Energy Content59
8.	Fatty Acid Analysis
9.	Amino Acid Analysis67
10.	Vitamin Analysis71
11.	Biological Methods for Feed and Fodder Evaluation79
12.	Enteric Methane Measurement in Ruminants89
13.	Rumen Liquor Analysis97
14.	Assessment of Nutritional Quality of Silage113

15.	Detection of	f Adulterants in Animal Feed	119
16.	Detection of	f Anti-Nutritional Factors in Feed	131
17.	Determinati	on of Pesticide Residues	161
	References.	Managaran Managa	165
	Appendice	s	
	Appendix-I:	List of abbreviations	169
	Appendix-II:	Nutrient composition of common feedstuffs	172
		Units and conversion	174
		Frequently used standards of concentration	176

1

# **Preparation and Expression of Standard Solution**

1.1.	Standard solution
1.2.	Expressing concentration of standard solution1
1.3.	Dilution4
1.4.	Indicator 5
1.5.	Acid-base titration
1.6.	Preparation of standard solution commonly used in nutritional analysis

### 1.1. Standard solution

A solution of known concentration is called the standard solution. A primary standard is a reagent that is extremely pure and stable; it not a hydrate/it has no water of hydration, and it has a high molecular weight. A secondary standard is a standard that is prepared in the laboratory for a specific analysis. It is usually standardized against a primary standard. The term secondary standard can also be applied to a substance whose active agent contents have been found by comparison against a primary standard.

### 1.2. Expressing concentration of standard solution

The concentration of a solution can be expressed in the following ways.

### i) Molarity (M)

It is defined as the number of gram moles of solute dissolved in one litre of the solution.

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### **Appendices**

### **APPENDIX I**

### LIST OF ABBREVIATIONS

%	Percentage	IU	International unit
°C	Degree celsius	IVGPT	<i>In vitro</i> gas production technique
μg	Micro gram	kg	Kilo gram
μl	Micro litre	1	Litre
AAS	Atomic absorption spectrophotometry	L-BAPA	Benzoyl-L-arginine-p- nitroanilide
ADF	Acid detergent fibre	M	Molarity
ADFCP	Acid detergent fibre crude protein	m	Molality
ADIN	Acid detergent insoluble nitrogen	MBP	Microbial biomass production
ADL	Acid detergent lignin	Mcal	Mega calorie
ADS	Acid detergents solution	ME	Metabolizable energy
AIA	Acid insoluble ash	mg	Milligram
ANFs	Anti-nutritional factors	mm	Millimeter
AOAC	Association of Official Analytical Chemists	mol	Mole
BCAA	Branched chain amino acids	Mol. wt.	Molecular weight

A	pendic	es
	p =cite.	

ВСРІ	Bromocresol purple index	N	Normality
BIS	Bureau of Indian Standard	NDF	Neutral detergent fibre
BSA	Bovine serum albumin	NDS	Neutral detergent solution
CC	Cubic centimeters	NFE	Nitrogen free extract
CF	Crude fibre	ng	Nano gram
$CH_4$	Methane	$NH_3-N$	Ammonia nitrogen
CP	Crude protein	NIR	Near-infrared
DC	Digestibility coefficient	NPN	Non protein nitrogen
DE	Digestible energy	OM	Organic matter
DM	Dry matter	pg	Pico gram
EE	Ether extract	ppb	Parts per billion
ECD	Electron capture detector	ppm	Parts per million
ELISA	Enzyme-linked immunosorbent assays	RUSITEC	Rumen simulation technique
Eq. wt.	Equivalent weight	SAA	Sulphur amino acids
FAAS	Flame atomic absorption spectrophotometry	SF <sub>6</sub>	Sulphur hexafluoride
FAMEs	Fatty acid methyl esters	TCA	Trichloroacetic acid
g	Gram	TDDM	True degradability of dry matter
G	Gauge	TDN	Total digestible nutrients
GC	Gas chromatography	TDOM	True degradability of organic matter
GE	Gross energy	TIA	Trypsin inhibitor activity
GFAAS	Graphite furnace atomic absorption spectrometry	TLC	Thin layer chromatography
GHG	Green house gas	TMR	Total mixed ration
GLC	Gas liquid chromatography	v/v	Volume/volume

HPLC	High performance liquid chromatography	VFA	Volatile fatty acid
hr(s)	Hour(s)	w/v	Weight/volume
ICP-AES	Inductively coupled plasma-atomic emission spectrometry	w/w	Weight/weight
ISO	International Standard Organization		

**APPENDIX II** 

NUTRIENT COMPOSITION OF COMMON FEEDSTUFF

Keal/         protein bring         Crude bring         Ethic bring         Lysine bring         Ametrino bring         Calcului bring           kg)         (%)         (%)         nine         (%)           kg)         (%)         (%)         nine         (%)           kg)         (%)         nine         (%)           kg)         (%)         nine         (%)           3309         9.2         3.0         3.8         0.18         0.15         0.25           2950         12.7         4.0         3.0         0.43         0.20         0.13           2345         12.7         4.0         2.5         0.47         0.20         0.19           product         2345         7.9         8.0         2.5         0.47         0.20         0.29           ce polish         2135         14.1         14.0         1.0         0.44         0.31         0.35           protein         2387         12.7         10.9         3.0         0.53         0.09         0.19           protein         2300         45.0         6.0         1.0         2.5         0.76         0.36           reake         2700         <	* * * * * * * * * * * * * * * * * * *		-	-	7.01		Martin	1.00	Dhoga
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by product         2345         7.9         8.0         2.5         0.33         0.17         0.11           by product         12.0         8.0         2.5         0.33         0.17         0.11           lish         2837         12.7         12.0         16.0         0.55         0.24         0.27           d rice polish         2135         14.1         14.0         1.0         0.44         0.31         0.27           bran         1286         17.2         10.9         3.0         0.53         0.09         0.19           bran         n meal         2300         45.0         6.0         1.0         2.57         0.76         0.36           s oxybean         3275         36.5         5.5         18.0         2.35         0.53         0.25           Inut cake         2700         38.0         9.0         8.0         1.6         0.42         0.16           inut ext.         2128         40-42         11.2         1.0         1.0         0.60         0.31	Wheat	3045	10.3	3.0	2.5	0.47	0.21	0.19	1.12
by product         2618         12.0         0.60         0.20         0.29           lish         2837         12.7         12.0         16.0         0.55         0.24         0.27           brain         2135         14.1         14.0         1.0         0.44         0.31         0.37           brain         1286         17.2         10.9         3.0         0.53         0.09         0.19           brain         1286         17.2         10.9         3.0         0.53         0.09         0.19           brain         3200         45.0         6.0         1.0         2.57         0.76         0.36           i soybean         3275         36.5         5.5         18.0         2.35         0.53         0.25           inut cake         2700         38.0         9.0         8.0         1.6         0.42         0.16           inut ext.         2128         40-42         11.2         1.0         1.47         0.68         0.31           wer cake         1600         28.0         1.0         1.0         0.60         0.37	Rice	2345	7.9	8.0	2.5	0.33	0.17	0.11	0.48
2837 12.7 12.0 16.0 0.55 0.24 0.27  h 2135 14.1 14.0 1.0 0.44 0.31 0.37  1286 17.2 10.9 3.0 0.53 0.09 0.19  2300 45.0 6.0 1.0 2.57 0.76 0.36  3275 36.5 5.5 18.0 2.35 0.53 0.25  2700 38.0 9.0 8.0 1.6 0.42 0.16  2128 40-42 11.2 1.0 1.47 0.68 0.31  1600 28.0 26.0 1.0 1.00 0.60 0.37	Barely	2618	12.0			09.0	0.20	0.29	0.64
2837 12.7 12.0 16.0 0.55 0.24 0.27  bolish 2135 14.1 14.0 1.0 0.44 0.31 0.37  tein 1286 17.2 10.9 3.0 0.53 0.09 0.19  thin 2300 45.0 6.0 1.0 2.57 0.76 0.36  ke 2700 38.0 9.0 8.0 1.6 0.42 0.16  t. 2128 40-42 11.2 1.0 1.47 0.68 0.31  te 1600 28.0 26.0 1.0 1.00 0.60 0.37	Cereal by product								
volish         2135         14.1         14.0         1.0         0.44         0.31         0.37           tein         2300         45.0         6.0         1.0         2.57         0.76         0.36           un         3275         36.5         5.5         18.0         2.35         0.53         0.25           ke         2700         38.0         9.0         8.0         1.6         0.42         0.16           t.         2128         40-42         11.2         1.0         1.47         0.68         0.31           e         1600         28.0         26.0         1.0         1.00         0.60         0.37	Rice polish	2837	12.7	12.0	16.0	0.55	0.24	0.27	1.37
tein         1286         17.2         10.9         3.0         0.53         0.09         0.19           th         2300         45.0         6.0         1.0         2.57         0.76         0.36           th         3275         36.5         5.5         18.0         2.35         0.53         0.25           ke         2700         38.0         9.0         8.0         1.6         0.42         0.16           t.         2128         40-42         11.2         1.0         1.47         0.68         0.31           te         1600         28.0         26.0         1.0         1.00         0.60         0.37	De-oiled rice polish	2135	14.1	14.0	1.0	0.44	0.31	0.37	1.80
tein     2300     45.0     6.0     1.0     2.57     0.76     0.36       un     3275     36.5     5.5     18.0     2.35     0.53     0.25       ke     2700     38.0     9.0     8.0     1.6     0.42     0.16       t.     2128     40-42     11.2     1.0     1.47     0.68     0.31       e     1600     28.0     26.0     1.0     1.00     0.60     0.37	Wheat bran	1286	17.2	10.9	3.0	0.53	60.0	0.19	1.12
th 3275 36.5 5.5 18.0 2.57 0.76 0.36 c.36 ke 2700 38.0 9.0 8.0 1.6 0.42 0.16 c.31 c. 1600 28.0 26.0 1.0 1.00 0.60 0.37	Vegetable protein								
th 3275 36.5 5.5 18.0 2.35 0.53 0.25 ke 2700 38.0 9.0 8.0 1.6 0.42 0.16 t. 2128 40-42 11.2 1.0 1.00 0.60 0.37 e. 1600 28.0 26.0 1.0 1.00 0.60 0.37	Soybean meal	2300	45.0	0.9	1.0	2.57	0.76	0.36	0.90
2700     38.0     9.0     8.0     1.6     0.42     0.16       2128     40-42     11.2     1.0     1.47     0.68     0.31       1600     28.0     26.0     1.0     1.00     0.60     0.37	Full fat soybean	3275	36.5	5.5	18.0	2.35	0.53	0.25	0.58
2128 40-42 11.2 1.0 1.47 0.68 0.31 1600 28.0 26.0 1.0 1.00 0.60 0.37	Groundnut cake	2700	38.0	0.6	8.0	1.6	0.42	0.16	0.16
1600 28.0 26.0 1.0 1.00 0.60 0.37	Groundnut ext.	2128	40-42	11.2	1.0	1.47	89.0	0.31	29.0
	Sunflower cake	1600	28.0	26.0	1.0	1.00	09.0	0.37	0.30

De-oiled mustard cake       1672       31.7       11.6       2.3       1.37         Cotton seed meal       1556       25.9       21.3       0.9       1.07         Guar meal       1821       37.1       10.5       6.7       2.58         Maize gluten meal       2732       28.2       -       2.5       1.02         Brewer's dried grains       2732       28.2       -       0.98         Animal proteins       1834       43.1       2.5       6.0       2.5         Meat meal       2300       56.2       2.6       6.8       4.00         Meat cum bone meal       2100       48.0       8.0       3.72         Mineral source       200       2.5       6.0       2.5		23/3	35.1	9.6	8.0	1.14	0.42	0.89	1.78
1556 25.9 21.3 0.9 1821 37.1 10.5 6.7 1 3300 60.0 2.0 2.5 ains 2732 28.2		1672	31.7	11.6	2.3	1.37	0.51	0.58	0.82
1821 37.1 10.5 6.7 ains 2732 28.2 - 2.5 1834 43.1 2.5 6.0 2300 56.2 2.6 6.8 eal 2100 48.0 - 8.0	Cotton seed meal	1556	25.9	21.3	6.0	1.07	0.41	0.52	98.0
ains 2732 28.2 - 2.5  ains 2732 28.2 - 2.5  1834 43.1 2.5 6.0  2300 56.2 2.6 6.8  eal 2100 48.0 8.0	Guar meal	1821	37.1	10.5	6.7	2.58	0.39	0.41	0.50
ains 2732 28.2	Maize gluten meal	3300	0.09	2.0	2.5	1.02	1.28	0.22	0.19
1834 43.1 2.5 6.0 2300 56.2 2.6 6.8 500e meal 2100 48.0 8.0  urce	Brewer's dried grains	2732	28.2	ä	Ī	0.98	0.43	0.29	0.54
1834 43.1 2.5 6.0 2300 56.2 2.6 6.8 500 56.2 2.6 6.8 aurce 8.0	Animal proteins								
2300 56.2 2.6 6.8 50ne meal 2100 48.0 8.0 wrce	Fish meal	1834	43.1	2.5	0.9	2.5	1.08	7.16	2.5
oone meal 2100 48.0 8.0 wrce	Meat meal	2300	56.2	2.6	8.9	4.00	0.84	2.68	2.06
urce		2100	48.0	100	8.0	3.72	0.75	11.25	5.39
DCP	Mineral source								
	DCP	10	5000	ř.	16	ë	į	23	18
1009	Limestone	0	1146	ë	10	ž	*	35.0	28
Oyster shell	Oyster shell	Oi.	3	11/2	E	Ē	Ř	38.0	а

### UNITS AND CONVERSION

Unit	Description	Conversion	Remarks
Pressur	e		
Pa	Pascal	Standard unit	Pascal = Newton/ m <sup>2</sup>
atm	Atmosphere (physical)	101,325 Pa	Pressure in gases
atm	Atmosphere (technical)	98,066.5 Pa	One atmosphere =1 kp/cm <sup>2</sup>
bar	Bar	100,000 Pa	Pressure in gas and liquid
psi	Pounds per Square Inch	6,894.757293168 Pa	1 psi equivalent 14.5 bar
mbar	Millibar	100 Pa	Equivalent one hector pascal(hPa)
	Millimeter-mercury column	133.32 Pa	
Energy			
Unit	Description	Conversion	
J	Joule	Standard unit	
BTU	British Thermal Unit	1055.058138 J	
cal	calorie	4.186794846 J	
HPh	Horsepower hour	2684517.413 J	
Wh	Watt hour	3599.9982 J	
Area		0: 1 1 ::	
m <sup>2</sup>	Square meter	Standard unit	
ha	Hectare	10,000 m <sup>2</sup>	
ft <sup>2</sup>	Square foot	0.0929 m <sup>2</sup>	
km²	Square kilometer	1,000,000 m <sup>2</sup>	
mi <sup>2</sup>	Square mile	2,589,988.11 m <sup>2</sup>	
Volum		Standard unit	
1-1 1-1-1	Liter	159 l	
bl, bbl	Barrel	4.55 l	
gal	Gallon (UK)	3.7862 I	
gal	Gallon (US)	0.4733 1	
pt	Pint	0.4733 I 0.9466 I	
qt	Quart	U.7 <del>4</del> 00 1	
Speed			

m/s	meters per second	Standard unit	
km/h	kilometers per hour	0.278 m/s	
mi/h	miles per hour	0.447 m/s	
Power			
N	Newton	Standard unit	
W	Watt	Standard unit	
hp	horsepower	745.701 W	
Length	ı		
m	meter	Standard unit	
ft	foot	0.3048 m	
In	Inch	0.0254 m	
mi	mile	1609.344 m	
yd	yard	0.9144 m	
Mass			
kg	kilogram	Standard unit	
lb	pound	0.4536 kg	
t	ton	1000 kg	
oz	ounce	0.02834 kg	
Temper	ature		
K	Kelvin	Standard unit	
°C	Degree Celsius	K = °C+273.15	
°F	Degree Fahrenheit	$^{\circ}F = 1.8 \times (K-273.15) + 32$	
		$^{\circ}C = \frac{(^{\circ}F - 32)}{1.8}$ $^{\circ}F = (^{\circ}C \times 1.8) + 32$	
Time		( = ==== )	
s	second	Standard unit	
m, min		60 s	
h	hour	3600 s	
d	day	86400 s	

## FREQUENTLY USED STANDARDS OF CONCENTRATION

Measurement	Notation	Generic formula	Typical units
Mass percentage	wt% or w/w%	g solute x 100/ g solution	%
Mass-volume percentage	m/v%	g solute x100/ml solution	% though strictly % g/ml
Volume-volume percentage	vol% or v/v%	ml solute x 100/ ml solution	%
Molarity	M	moles solute/ liter solution	mol/l (or M or mol/dm³
Molinity	TEI	moles solute/ kg solution	mol/kg
Molality	m	moles solute/ kg solvent	mol/kg (or m)
Molar fraction	(chi)	moles solute/ moles solution	(decimal)
Formal	F	moles undissolved solute/liter solution	mol/l (or F)
Normality	N	gram equivalents/ liters solution	N
Parts per hundred	%(or pph)	Dekagrams solute/ kilograms solution	dg/kg
Parts per thousand	%(or ppt)	grams solute/ kilograms solution	g/kg
Parts per million	ppm	mg solute/kg solution	mg/kg
Parts per billion		μg solute/kg solution	
Parts per trillion		ng solute/kg solution	ng/kg