Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/April-2014/14.pdf

RESEARCH ARTICLE
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The evaluation of metabolizable protein content of some indigenous feedstuffs used in ruminant nutrition

Lalatendu Keshary Das[1], S. S. Kundu[2], Dinesh Kumar[3] and Chander Datt[2]

1. Veterinary Dispensary, Kalampur, Kalahandi, Odisha, India;
2. Division of Dairy Cattle Nutrition, National Dairy Research Institute, Karnal, Haryana, India;
3. Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India.
Corresponding author: Lalatendu Keshary Das, email: drlalatendu27@gmail.com
Received: 01-02-2014, Revised: 20-03-2014, Accepted: 25-03-2014, Published online: 21-04-2014

doi: 10.14202/vetworld.2014.257-261
How to cite this article: Das LK, Kundu SS, Kumar D and Datt C (2014) The evaluation of metabolizable protein content of some indigenous feedstuffs used in ruminant nutrition, *Veterinary World* 7(4): 257-261.

Abstract

Aim: To determine the metabolizable protein (MP) content of common indigenous feedstuffs used in ruminant nutrition using *in situ* method.

Materials and Methods: Nine ruminant feeds such as maize grain (MG), groundnut cake (GNC), mustard oilcake (MOC), cottonseed cake (CSC), deoiled rice bran (DORB), wheat bran (WB), berseem fodder (BF), maize fodder (MF) and sorghum fodder (SF) were included in this study. Each test feed was dried, ground and chemically analysed for proximate principles (DM, CP, EE, OM, Total ash), fiber fractions (NDF, ADF, cellulose, hemicellulose, lignin), NDICP and ADICP. Two adult fistulated bulls were used for evaluating the protein degradation characteristics of each test feed using the nylon bag method. Metabolizable energy (ME) content of the test feeds were predicted from their chemical composition data using summative approach of NRC (2001) model. The equations of AFRC (1992) were used to predict the rumen degradable protein (RDP), digestible microbial protein (DMP), digestible undegraded feed protein (DUP) and MP content of test feeds.

Results: The MP content of MG, GNC, MOC, CSC, DORB, WB, BF, MF and SF was found to be 95.26, 156.41, 135.21, 125.06, 101.68, 107.11, 136.81, 72.01 and 76.65 g/kg DM, respectively. The corresponding ME (MJ/kg DM) content of the test feeds was 13.66, 13.12, 13.65, 10.68, 9.08, 11.56, 9.64, 8.33 and 8.03, respectively. Among the test feeds, GNC contained the highest and MF contained the lowest MP per kg DM.

Conclusion: It was concluded that the degradability of crude protein (CP) of the test feeds can be used in MP determination and diet formulation. Feed CP content is not available as such at intestinal level in ruminants as a definite part of it undergoes extensive microbial degradation in rumen. The pattern and extent of such degradation do influence the amount of protein presented to lower digestive tract (MP) for absorption and utilization in ruminants. It was also found that the MP content of a feed is not constant as that of its CP content as it entirely depends on the degradation characteristics in a given animal.

Keywords: *in situ* method, metabolizable energy, metabolizable protein, microbial protein, ruminant nutrition.

Introduction

In ruminants, ration balancing based on CP content of feedstuffs has been considered as obsolete due to the activities of ruminal microorganisms such as degradation of feed protein, synthesis of microbial protein and passage of both undegraded feed protein and microbial protein to lower intestine. Therefore protein evaluation system in ruminants is now based on RDP and RUP. Mere classification of feed protein into RDP and RUP also could not solve the purpose of balancing ruminant diets for adequate protein as it did not provide any information regarding the protein available to the animals in the lower intestinal tract for productive purposes. In such a situation, the concept of MP content of ruminant feeds seems more logical and scientific as it represents the actual quantity of true protein or amino acids that gets absorbed by the animal [1]. MP systems define the animal's requirement using estimates of available microbial and dietary escape protein [1, 2] and are potentially more accurate than the CP systems are.

The CP degradability can be measured by *in vivo* and *in vitro* methods. *In vitro* methods are quicker for screening of large number of feeds but do not give protein degradability in absolute terms. *In situ* method of Agricultural Research Council (ARC) using nylon bag is widely accepted to measure the degradability, which is analysed by a computer model developed by Orskov and McDonald [3]. Several attempts were made to estimate the MP content of animal feeds by various methods. Islam *et al.* [4] evaluated MP content of whole crop rice silage at different stages of harvest, which is usually not a common ruminant feed under Indian conditions. Similarly MP content of Wolffia meal was evaluated by Pooponpan *et al.* [5] in broilers. Rahbarpour *et al.* [6] used the nylon bag (*in situ*) technique to determine the MP value of tomato pomace. However MP values of typical ruminants feeds are lacking. It is important to note that, MP content of feeds is a better indicator of animal performances, (particularly dairy animal production) than the corresponding CP

Table-1: The chemical composition of feedstuffs (% DM)

Feedstuffs	DM	CP	EE	TA	NFC	NDF	ADF	ADL	NDICP	ADICP
MG	92.73	10.28	5.39	2.30	59.38	24.62	7.04	2.07	1.97	0.45
GNC	93.05	40.66	7.59	6.13	24.62	23.67	18.30	4.19	2.68	0.86
MOC	90.80	38.24	7.87	4.78	30.11	22.16	18.46	3.37	3.16	2.02
CSC	92.13	26.00	8.00	4.58	12.76	50.91	37.34	8.50	2.26	1.23
DORB	91.10	13.47	1.66	9.47	33.72	47.93	17.64	6.39	6.30	2.53
WB	88.62	15.68	2.92	5.48	40.17	39.86	11.48	2.84	4.11	0.68
BF	14.70	18.18	2.91	9.06	33.84	41.10	21.23	6.49	5.09	1.60
MF	14.94	9.77	1.55	9.25	17.22	65.36	35.36	4.58	3.16	1.52
SF	18.01	9.89	1.61	10.05	12.96	68.99	40.19	4.61	3.51	1.47

DM: Dry Matter, CP: Crude Protein, EE: Ether Extract, TA: Total Ash, NFC: Non Fibrous Carbohydrate, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, NDICP: Neutral Detergent Insoluble Crude Protein, ADICP: Acid Detergent Insoluble Crude Protein

values as evidenced by several research works. [7-10].

This study is, therefore, aimed at evaluating the ME and MP content of test feeds so that a database regarding the MP values of common indigenous ruminant feeds could be developed.

Materials and Methods

Ethical approval: The experimental design and plan of the present study was duly approved by the Institution Animal Ethics Committee of National Dairy Research Institute (NDRI), Karnal, Haryana.

Sample collection: The samples of feeds and fodders were collected from forage section of NDRI, Karnal and from local market of Karnal. Fodder samples were collected from at least five different areas within each field. They were thoroughly mixed and a composite sample of about 100 g was used. Similarly the concentrate feeds were collected from at least five different localities. The samples were dried in hot air oven at 60^0C for days till a constant weight was attained to ensure proper and complete drying of samples. The dried samples were ground through 1mm sieve using electrically operated willey mill. The ground samples were stored in bottles of 200 ml capacity, labeled properly and stored for further analysis.

Selection and feeding of fistulated animals: Two adult fistulated bulls were selected from institute herd (NDRI). They were fed a diet containing 40% concentrate, 40% green berseem and 20% wheat straw. The feed was offered in equal proportion for every 8 h to maintain a relatively constant rumen microbial environment.

In situ rumen incubation of feeds: Samples of total dry matter, approximating 5 g were placed into nylon bags (length 150x90 mm, 44 µm pore diameter and 350 mesh size) separately. Roughages were placed into nylon bags in 7 replicates and concentrates placed in 6 replicates. The bags were tied with nylon threads. Each sample was taken in quadripulates and experiment was repeated twice. The bags were tied to an iron chain and then suspended in the rumen of fistulated animals. Then the bags were taken out at 0, 2, 4, 8, 16, 24, 48 and 72 h interval for roughages and 0, 2, 4, 8, 16, 24 and 48 h interval for concentrates. The bags were then washed thoroughly under running tap water until clear water came out of the bags. The bags were then dried in hot air oven at 80^0C to a constant weight for 48 h to have properly dried feed samples after their exposure to ruminal degradation process. The residual dry matter (DM) at different incubation periods was taken and analyzed for CP disappearance.

Chemical analysis: DM, organic matter (OM), CP, ether extract (EE) and total ash in test feed samples were determined as per methods prescribed by AOAC [11]. The fractions of cell wall constituents neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as per methods of Van Soest *et al.* [12]. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were estimated as per Licitra *et al*. [13]. Total digestible nutrients (TDN) and ME content of feeds were predicted from their chemical composition values as per NRC [14]. DM and CP degradation kinetics were evaluated by the model of Orskov and McDonald [3]. MP content of the test feeds were estimated using the equations of AFRC [15]. The ruminal outflow rate of feed DM and CP were assumed to be 5%/h as per Ramirez et al. [16]. The factor 'y' for calculating the microbial yield was taken as 10 for growing animal as per AFRC.

Statistical analysis: The results obtained were analyzed using software package SPSS version 16.0 [17]. Means were compared using one way analysis of variance (ANOVA) test at 5% level of significance.

Results and Discussion

Chemical composition: The chemical composition of test feeds is presented in Table-1. As expected, the oil cakes i.e. GNC, MOC and CSC contained higher CP, while among green fodders, berseem had highest CP because of its leguminous nature. CSC, DORB and WB contained higher NDF and ADF among concentrate feeds, while MF and SF recorded higher NDF and ADF among green fodders. The obtained chemical compositions of test feeds are different compared to reports of NRC [14] and AFRC [15]. These differences may be related to variance in environmental conditions, variety of feeds, maturity during time of cut, oil extract assay and other processing factors.

TDN, ME and fermentable ME (FME) content: The

Table-2: TDN, ME and FME content of feedstuffs

Feedstuffs	td NFC (% DM)	td CP (% DM)	td FA (% DM)	td NDF (% DM)	TDN (% DM)	DE (MJ/kg)	ME (MJ/kg)	FME (MJ/kg)
MG	58.19	10.10	4.39	12.30	83.48	15.38	13.66	11.77
GNC	24.13	40.31	6.59	8.30	80.57	14.85	13.12	10.46
MOC	29.51	37.43	6.87	8.02	83.42	15.37	13.65	10.90
CSC	12.51	25.51	7.00	20.71	67.48	12.44	10.68	7.88
DORB	33.11	12.45	0.66	18.85	58.91	10.86	9.08	8.50
WB	39.36	15.41	1.92	20.13	72.23	13.31	11.56	10.54
BF	33.16	16.36	1.91	15.08	61.90	11.41	9.64	8.62
MF	16.87	8.11	0.55	35.64	54.85	10.11	8.33	7.79
SF	12.70	8.27	0.61	37.89	53.24	9.81	8.03	7.47

td: Truly Digestible, FA: Fatty Acid, TDN: Total Digestible Nutrient, DE:Digestible Energy, ME: Metabolizable Energy, FME: Fermentable Metabolizable Energy.

Table-3: *In situ* DM disappearance (%) of feedstuffs

Feeds	Period of incubation (h)							
	0	2	4	8	16	24	48	72
MG	35.20	42.24	46.58	54.82	68.43	75.49	83.78[b]	-
GNC	62.44	71.81	76.01	78.77	83.02	85.77	88.64a[b]	-
MOC	59.51	74.60	82.16	83.47	85.74	90.26	92.19[a]	-
CSC	32.06	41.46	49.01	54.43	61.09	63.48	66.11[d]	-
DORB	30.98	51.08	53.82	58.76	61.93	68.47	74.20[c]	-
WB	38.78	53.13	55.83	62.12	67.46	69.43	73.33[c]	-
BF	33.76	44.62	46.70	51.30	55.21	70.54	77.06	78.60[a]
MF	13.08	18.92	21.35	28.92	43.43	48.08	57.43	60.88[b]
SF	17.27	25.87	28.58	33.17	38.04	46.50	48.71	59.15[b]

Means bearing different superscripts in the same column differs significantly ($P < 0.05$). 48 h DM degradability of concentrate feeds and 72 h DM degradability of green fodders have been compared.

Table-4: *In situ* CP disappearance (%) of feedstuffs

Feeds	Period of incubation (h)							
	0	2	4	8	16	24	48	72
MG	42.14	48.53	62.99	71.25	78.74	82.20	88.46[a]	-
GNC	35.97	40.33	50.89	66.44	83.06	89.33	92.07[a]	-
MOC	28.23	57.56	71.66	76.36	80.51	82.14	84.02[a]	-
CSC	32.19	39.39	43.12	58.56	62.56	73.14	89.74[a]	-
DORB	17.19	21.25	28.63	33.78	39.72	55.08	62.93[b]	-
WB	38.46	51.45	54.54	61.10	67.56	77.70	85.56[a]	-
BF	7.09	9.83	17.64	26.73	42.62	62.38	69.24	73.88[a]
MF	22.22	29.24	41.26	61.23	64.44	69.40	71.66	76.73[a]
SF	11.74	21.59	36.85	53.81	57.61	63.53	66.44	68.70[b]

Means bearing different superscripts in the same column differs significantly ($P < 0.05$). 48 h CP degradability of concentrate feeds and 72 h CP degradability of green fodders have been compared.

values for TDN, ME and FME content of test feeds along with their digestible nutrients are presented in Table-2. MG, GNC and MOC contained higher TDN and ME values among concentrate feeds, while among green fodders berseem had higher TDN and ME content. Similar trend was observed in FME content of feedstuffs. Nutrient composition of feeds as enlisted in NRC [14] and feed library of CNCPS [18] reported similar TDN and ME values. However, NRC [14] reported less TDN value for MOC than the present findings. CNCPS [18] reported higher value of TDN for CSC. Both NRC [14] and CNCPS [18] reported slightly higher TDN values for green fodders than the present results. Such differences in feed chemical composition again can be attributed to the variations in environmental conditions and soil status in which they were grown.

In situ DM and CP disappearance: The disappearances of DM and CP at different time of incubation are shown in Table-3 and 4. Among concentrate feeds, GNC had highest (62.44%) zero h DM degradability followed by MOC (59.51%). Among forages, berseem fodder had higher (33.76%) zero h DM degradability. MOC recorded highest (92.19%) 48 h DM degradability, while CSC recorded lowest (66.11%) among concentrate feeds. Berseem green recorded highest (78.60%) 72 h DM degradability. The results for concentrate feeds are in accordance to the findings of Singh and Kundu [19]. Sampath et al. [20] reported similar values of maize fodder. Among concentrate feeds, maize grains had

Table-5: CP degradation kinetics of feedstuffs

Feeds	a	b	c	RDP (% CP)	RUP (% CP)	RDP (% DM)	RUP (% DM)	ERDP (g/kg DM)
MG	41.66	44.59	0.129	73.79	26.21	7.59	2.69	67.30
GNC	32.83	61.44	0.097	73.37	26.63	29.83	10.83	271.63
MOC	28.40	53.22	0.393	75.61	24.39	28.91	9.33	267.42
CSC	33.89	62.08	0.045	63.30	36.70	16.46	9.54	146.95
DORB	17.75	51.89	0.044	42.04	57.96	5.66	7.81	51.84
WB	42.50	44.30	0.064	67.37	32.63	10.56	5.12	92.31
BF	4.18	71.90	0.053	41.18	58.82	7.49	10.69	73.34
MF	20.23	53.04	0.136	59.01	40.99	5.77	4.00	53.70
SF	10.44	55.89	0.152	52.50	47.50	5.19	4.70	49.85

a: quickly degradable fraction, b: slowly degradable fraction, c: degradation rate of fraction b, RDP: Rumen degradable protein, RUP: Rumen undegradable protein, ERDP: Effective rumen degradable protein

Table-6: Metabolizable protein content of feedstuffs

Feeds	MCP (g/kg DM)	DMP (g/kg DM)	DUP (g/kg DM)	MP(g/kg DM)
MG	117.74	75.06	20.20	95.26
GNC	104.64	66.70	89.71	156.41
MOC	108.96	69.46	65.75	135.21
CSC	78.80	50.24	74.82	125.06
DORB	84.99	54.18	47.50	101.68
WB	105.38	67.18	39.93	107.11
BF	86.22	54.96	81.85	136.81
MF	77.88	49.65	22.36	72.01
SF	74.67	47.60	29.05	76.65

MCP: Microbial crude protein, DMP: Digestible microbial protein, DUP: Digestible undegraded feed protein, MP: Metabolizable protein

highest (42.14%) zero h CP degradability followed by wheat bran (38.46%). Among forages, maize fodder had higher (22.22%) zero h CP degradability. 48 h CP degradability was highest (92.07%) in GNC and lowest (62.93%) in DORB among concentrate feeds. Among forages, both berseem and maize fodder recorded higher (73.88 and 76.73%) 72 h CP degradability.

CP degradation kinetics: CP degradation parameters (a, b and c) along with RDP, RUP and effective RDP (ERDP) content of test feeds are presented in Table-5. The RDP content was highest (75.61%) in MOC and lowest (42.04%) in DORB among concentrate feeds. Among forages, maize fodder had highest (59.01%) RDP value, while berseem had the lowest (41.18%) value. CP degradation rate was highest (0.393) for MOC and lowest (0.044) for DORB among concentrate feeds. Among forages, both maize and sorghum fodder had higher (0.136 and 0.152) CP degradation rates. Wadhwa *et al.* [21] and Singh and Kundu [19] reported similar CP degradation kinetics of concentrate feeds. GNC and MOC recorded higher ERDP content, while DORB contained lower ERDP.

MP content: MP content of test feeds along with their DMP and DUP content are presented in Table-6. There were differences between obtained results for DUP and MP of test feeds compared to AFRC [15]. The differences are possibly due to variations in microbial yield, type, variety and processing of feedstuffs, soluble and insoluble protein content etc. GNC showed high MP compared to other feeds that can be resulted from high DUP content resulting supply of high amino acids for ruminants. High MP content of berseem as compared to other fodders can be attributed to the same factor as that of GNC. Lower MP values of MG, MF and SF may be due to their lower DUP content.

Conclusion

It is concluded that feed protein fractions vary considerably from each other with respect to their degradation kinetics in rumen; therefore only CP content of a feed can not represent its true feeding value in ruminants. CP value of a feed is always constant as it is a chemical composition value and thus will be same for all class of animals whether ruminants or non ruminants. But the degradation kinetics of feed CP content will be different from animal to animal in case of ruminants as a result the MP content of a given feed will be different in different animals. However as MP value represents the true protein amount available to ruminants for body utilization, it can be used in diet formulation for ruminants and thus is more reliable in predicting the feeding value.

Authors' contributions

SSK and CD designed the experiments. LKD and DK carried out the experimental work. LKD wrote the manuscript and did the data analysis. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to the Director, NDRI, Karnal, Haryana for providing financial support to carrying out this study. The corresponding author is indebted to NDRI and ICAR for providing him institutional scholarship during his M.V.Sc programme at NDRI (2010 -12).

Competing interests

The authors declare that they have no competing interests.

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