Single point ruminal incubation times necessary to estimate rumen degradable protein content in concentrate feeds

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Transl. Anim. Sci. 2019.3:1686–1690
doi: 10.1093/tas/txz058

INTRODUCTION

Accurate prediction of metabolizable protein (MP) supply and meeting the ruminal microbial demand for ammonia N are important to minimize feed costs and nitrogen (N) waste (NASEM, 2016; Valadares Filho et al., 2016). The in situ bag technique (Ørskov and McDonald, 1979; de Boer et al., 1987; Wilkerson et al., 1995; Mathis et al., 2001) assesses rumen degradable protein (RDP) and rumen undegradable protein (RUP) which can be used to calculate MP supply. This technique uses either a fixed ruminal incubation time of 16 h (Calsamiglia and Stern, 1995; Paz et al., 2014) or multiple incubation time points and mathematically models protein fractions (Ørskov and McDonald, 1979). For computing MP supply, the NASEM (2016) uses fixed values of RUP digestibility of 80% and 60% for concentrates and roughage, respectively, whereas BR-CORTE (Valadares Filho et al., 2016) recommends a fixed value of 80% for RUP digestibility of both concentrates and roughage. Improvements in estimating RDP and RUP of feeds would foster more accurate dietary formulations and potentially reduce the environmental N burden. Because feedstuffs vary widely in physical characteristics, nutrient composition, and potential ruminal degradability, we hypothesized that the single point incubation time necessary to best estimate RDP would vary between feeds. Therefore, our objective was to determine the optimal single point incubation time necessary to estimate RDP of 11 energy and protein concentrates.

MATERIALS AND METHODS

Characterization of Concentrate Samples

The experiment was carried out at the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. The procedures for the humane care and animal handling were in agreement with the ethical committee for the Animal Use in the Universidade Federal de Viçosa (protocol number 96/2014). Eleven types of concentrates were evaluated: 6 energy concentrates: wheat bran (*Triticum aestivum*), rice meal (*Oryza sativa*), ground corn (*Zea mays* L.), ground sorghum (*Sorghum vulgare*), ground corn cob (*Zea mays* L.), and soybean hulls (*Glycine max* (L.) *Merr*); and 5 protein concentrates: cottonseed meal (*Gossypium hirsutum*), soybean meal (*Glycine max* (L.) *Merr*), ground bean (*Phaseolus vulgaris* L.), peanut meal (*Arachis hypogaea* L.), and sunflower meal (*Helianthus annuss*).

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Received April 2, 2019.
Accepted June 4, 2019.

All samples were ground using a Wiley mill (TECNAL, Piracicaba, São Paulo, Brazil) with a 1-mm sieve for chemical analyses and a 2-mm sieve for ruminal in situ incubation. Chemical analyses included dry matter, organic matter, and N performed according to the AOAC (2012; method numbers 934.01, 930.05, and 981.10, respectively). Neutral detergent fiber (NDF) and NDF corrected for ash and protein analyses were performed according to techniques described by Mertens (2002) without the addition of sodium sulfite, but with the addition of thermostable alpha-amylase to the detergent. The chemical composition of feeds is available in Table 1.

Incubation Procedure

The 11 feeds were divided into four groups and ruminally incubated in four crossbred bulls in a 4 × 4 Latin square design. Of the four feed groups, three contained three different types of feedstuffs, and one group contained two feedstuffs, as the following: group 1 (ground sorghum, wheat bran, soybean meal), group 2 (sunflower meal, ground corn, ground bean), group 3 (rice meal, ground corn cob, peanut meal), and group 4 (cottonseed meal and soybean hulls). Within each period, each feed group was incubated in the rumen of a different bull. Nylon bags (Sefar Nitex; Sefar, Thal, Switzerland; porosity of 50 µm, 8 × 15 cm) were used and 6.0 g of previously prepared feed samples were quantitatively weighed and placed into each bag. Ruminal incubation times were 0, 2, 4, 8, 16, 24, 48, and 72 h. The number of bags used for each feed sample varied as a function of the time of incubation to obtain sufficient residue for laboratory analyses: 1 bag for 0 and 2 h, 2 bags for 4 and 8 h, 3 bags for 16 h, 4 bags for 24 h, and 5 bags for 48 and 72 h, for a total of 22 bags per feedstuff and 66 ruminally incubated bags per animal within period (excluding time 0).

In situ bags containing samples were attached to a steel chain (90 × 2 cm) with a weight at the end, thus allowing for complete immersion within the ruminal fluid, below the fiber mat. The bags were placed into the rumen in reverse order so that all bags were removed at the same time then washed in running water followed by washing in cold tap water by hand by the same person. The endpoint for washing was the high clarity of rinse water [adapted from Wanderley et al. (1993) and Machado et al. (2013)]. Nylon bags for time 0 were not incubated in the rumen but were included in the washing procedure with the incubated bags. After washing, bags were oven-dried at 55 °C for 72 h, after which they were placed in an oven at 105 °C for 2 h, placed in a desiccator, and finally weighed.

Statistical Analyses

Degradation profiles of crude protein (CP) were interpreted using the asymptotic model of Ørskov and McDonald (1979):

$$\text{CPdt} = a + b \times \left(1 - e^{(-\text{kd} \times t)}\right)$$

where CPdt = the percentage of CP degraded at time t; t = the effect of time on the variables (h); a = the soluble fraction of the CP (%); b = the

Table 1. Chemical composition of the feedstuffs used to estimate rumen degradable protein content

	Analyzed feed composition[1], % DM						
Feed	DM	OM	CP	NDF	NDFap	NDIP	NDIA
Energy concentrates							
Wheat bran	86.9	95.1	19.8	33.8	30.3	3.36	0.07
Rice meal	86.5	90.4	16.4	21.2	17.9	3.10	0.14
Ground corn	86.5	98.7	9.63	8.02	5.62	2.36	0.04
Ground sorghum	86.3	98.7	10.4	9.59	6.57	2.88	0.13
Ground corn cob	87.5	96.7	7.65	32.4	29.9	2.15	0.36
Soybean hulls	87.5	95.5	14.6	65.5	58.8	5.73	1.03
Protein concentrates							
Cottonseed meal	89.1	93.4	41.3	33.7	19.2	13.8	0.67
Ground bean	93.5	95.1	26.5	19.4	15.0	3.78	0.54
Soybean meal	87.9	93.7	52.5	20.2	18.6	1.47	0.13
Peanut meal	88.2	96.2	52.1	12.6	10.3	1.64	0.62
Sunflower meal	85.1	93.7	32.2	47.5	45.1	1.59	0.85

[1]DM = dry matter; OM = organic matter; NDFap = NDF corrected for ash and protein; NDIP = neutral detergent insoluble protein; and NDIA = neutral detergent insoluble ash.

insoluble fraction that is potentially degradable (%); and kd = the degradation rate of "*b*" (h^{-1}).

The RDP was calculated as follows:

$$RDP = a + b \times \frac{kd}{kd + kp}$$

where kp is the ruminal outflow rate (h^{-1}). The other terms were previously defined.

Two outflow rates (0.05 and 0.08 h^{-1}) were used to estimate RDP values and to estimate single point incubation times necessary to estimate RDP of the feeds used in this study (Habib et al., 2013; Steingass et al., 2013). A ruminal passage rate of 0.05 h^{-1} (medium rate) was used to simulate the passage rate for calves, low-milk yield dairy cows, and beef cattle, whereas 0.08 h^{-1} (high rate) was used to simulate the passage rate for high milk yield dairy cows according to the AFRC (1993).

The incubation time (*t*) necessary to estimate the RDP of each feed was quantified as the incubation time when the degraded fraction of CP becomes equal to the RDP estimate. The following equation was used:

$$t = -\ln\left[\frac{1 - (RDP - a/b)}{kd}\right]$$

In addition, aiming to identify concentrate subgroups with similar incubation times to estimate the RDP, the incubation times obtained for both passage rates were submitted to a multivariate nonhierarchical clustering procedure (Katthree and Naik, 2000) using the FASTCLUS procedure of SAS (version 9.4). All statistical procedures were conducted considering 0.05 as the critical level for the probability of type I error.

RESULTS AND DISCUSSION

The values of a, b, kd, and RDP for the two passage rates are available in a complementary study (Menezes et al., 2017). The CP degradation values are available in Table 2, and in accordance with Razzaghi et al. (2016), the ruminal degradability of protein was affected by the type of feed and chemical composition.

The cluster analysis allowed us to group the feeds into three subgroups (high-starch content, low-starch content, and protein concentrates) according to the single point incubation time needed to estimate RDP content (Table 2). We highlight that the overall R^2 of the clustering procedure was high ($R^2 = 0.944$).

Knowledge regarding RDP content of feeds is necessary to formulate diets to meet nutrient requirements of beef and dairy cattle. Ruminants have particularities with their protein nutrition because most of their amino acids and absorbable proteins (50% to 80%) are from microbial protein synthesized in the rumen (Bach et al., 2005). In this study, we evaluated

Table 2. Crude protein degradation and incubation time necessary to estimate rumen degradable protein of concentrate feeds used in cattle diets when considering two passage rates

	Concentrate feeds										
	Energetic high starch			Energetic low starch			Protein concentrate				
Time, h	Ground corn	Ground sorghum	Ground corn cob	Wheat bran	Rice meal	Soybean hulls	Cottonseed meal	Soybean meal	Ground bean	Peanut meal	Sunflower meal
Crude protein degradation, %											
0	33.77	21.60	17.83	30.54	37.61	22.12	32.64	25.84	24.99	30.77	35.88
2	50.56	42.41	39.33	63.21	70.43	51.78	59.85	54.25	40.68	64.58	68.01
4	51.79	44.02	44.75	75.42	70.74	56.73	63.09	59.84	46.8	61.15	74.12
8	54.25	46.32	47.25	89.57	75.46	65.64	78.43	73.44	61.83	73.08	81.16
16	63.55	48.53	57.66	92.48	80.53	76.44	85.98	89.83	77.72	87.01	89.33
24	76.76	55.52	65.11	94.63	86.73	81.56	90.59	97.01	82.55	91.43	92.93
48	94.66	79.86	86.22	95.29	87.57	86.30	95.87	98.59	97.48	97.82	96.13
72	96.92	88.05	91.43	95.16	88.36	91.37	96.19	98.55	99.45	98.54	96.58
Incubation time, h (cluster analysis)											
kp = 0.05 h^{-1}											
IIT[1]	15.2	16.3	14.8	6.20	6.10	7.90	9.10	9.20	11.4	9.80	10.2
AIT ± SEM[2]		15.4 ± 0.46			6.80 ± 0.60				9.90 ± 0.41		
kp = 0.08 h^{-1}											
IIT	10.3	10.6	10.2	5.00	4.90	6.20	7.00	7.00	8.30	7.40	7.60
AIT ± SEM		10.4 ± 0.12			5.40 ± 0.41				7.50 ± 0.25		

[1]IIT = individual incubation time.

[2]AIT ± SEM = average incubation time plus standard error of the mean.

the single point incubation time needed to estimate RDP content of each feed and identified concentrate subgroups with similar incubation times while considering two passage rates, 0.05 h^{-1} (medium rate) and 0.08 h^{-1} (high rate), according to the AFRC (1993).

According to the cluster analysis, the high-starch energy concentrates needed approximately 15 h (15.4 ± 0.46 h) of incubation to estimate the RDP content at kp equal to 0.05 h^{-1}, and 10.4 ± 0.12 h at a kp equal 0.08 h^{-1}, a time longer than for the other subgroups. This occurred because of the structural characteristics of starch and the interactions with other components, such as proteins or lipids (Svihus et al., 2005). The presence of a protein matrix around the starch reduces access to microorganisms and enzymes necessary to digest feeds. Moreover, corn and sorghum plant seeds that are commonly found in Brazil have a harder endosperm, which, therefore, indicates a greater binding between protein and starch (McAllister et al., 1990) that would require more time to degrade similar amounts of CP in the rumen than plant seeds with softer endosperms.

The low-starch concentrates required the lowest incubation time (6.80 ± 0.60 h at kp = 0.05 h^{-1}; 5.40 ± 0.41 h at kp = 0.08 h^{-1}). The third subgroup, which was composed of protein concentrates, yielded intermediate values for incubation time to estimate RDP (9.90 ± 0.41 h at kp = 0.05 h^{-1}; 7.50 ± 0.25 h at kp = 0.08 h^{-1}). Data from Paz et al. (2014) indicated that 16 h of ruminal incubation was a necessary step in the mobile nylon bag technique to assess RDP content and to subsequently estimate RUP content (de Boer et al., 1987). According to our data, 16 h of ruminal incubation is indicated only for the high starch-energy concentrate subgroup of ground corn, ground sorghum, and ground corn cob at a passage rate of 0.05 h^{-1}. For the other feeds evaluated in this study, 16 h of incubation can overestimate ruminal CP digestibility. Therefore, the majority of concentrate feeds may not need to be incubated for 16 h in the rumen because of the chemical composition, particle size, and passage rate, as they rapidly flow to the intestine. Thus, we present the incubation times in Table 2 as those needed to estimate RDP content of concentrate feeds.

IMPLICATION

Single point ruminal incubation times needed to effectively estimate RDP, and consequently, RUP differ depending on feed type. Consequently, the standard 16 h incubation may not always be the most effective incubation time. Values published herein are suggested as alternatives that should improve estimates of RDP and foster more accurate estimates of MP supply.

ACKNOWLEDGMENTS

This study was made possible by grants from National Council of Scientific and Technological Development-National Institute of Science and Technology in Animal Science/Ciência Animal and Foundation for Research Support of the State of Minas Gerais.

Conflict of interest statement. None declared.

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