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The Effects of Indigestible Neutral Detergent Fiber (iNDF) of Alfalfa Hay and Corn Silage on Ruminal Degradability of Ration Fiber in Sheep

Research Article

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ABSTRACT

Two experiments were carried out to evaluate the effects of indigestible neutral detergent fiber (iNDF) of ration for estimation of rumen degradability of fiber in sheep. In experiment 1, to determine feed iNDF, four ruminal fistulated Zel ewes were used. Three grams of alfalfa hay, corn silage, barley grain, wheat bran, ration 1 (including alfalfa hay and concentrate), and ration 2 (including corn silage and concentrate) in four replications were put in nylon bags and incubated in the rumen for 288 h. In experiment 2, the ruminal degradation of dry matter (DM), NDF, iNDF and potential digestible NDF (pdNDF) of alfalfa hay, corn silage, in both ration 1 and 2, were determined with *in situ* method using four ruminal fistulated Zel ewes. Rumen disappearance of DM at 0 and 2 hour incubation time and NDF at 96 hour incubation time in ration 2 were higher. The soluble, slowly degraded fractions, and rate of degradation in rations 1 and 2 were 28.93 and 25.93%; 50.40 and 46.26%; 2.36 and 3.19 %/h for DM; 11.81 and 11.74%; 61.37 and 46.32%, and 1.74 and 2.33 %/h for NDF, respectively. The iNDF content in alfalfa hay, corn silage, barley grain, wheat bran, ration 1 and 2 were 30.74 ± 3.02, 16.84 ± 2.14, 4.77 ± 1.48, 11.75 ± 2.29, 21.32 ± 0.77, and 11.42 ± 0.91% of DM, respectively. The results showed that despite the similarities in NDF content in both rations, difference in the iNDF content could affect on degradability in rumen.

KEY WORDS alfalfa hay, corn silage, indigestible neutral detergent fiber, rumen degradability, sheep.

INTRODUCTION

Fiber is a complex material whose digestibility varies due to forage species, forage variety, plant maturity and growing environment (Combs, 2013). The NDF fraction is a critical measure of fiber fraction, bulky, slow digestible component which can restrict intake, energy utilization, and feed quality (Raffrenato and Van Amburgh, 2010; Combs, 2013). The NDF is a non-uniform feed fraction and this is likely part of the reason for the many contradictions in the ration formulation about the effects of pdNDF on animal response. The NDF is not an ideal nutritive entity because it is not chemically identical and the proportion of cellulose, hemicellulose, and lignin varies among forage types, is affected by species, maturity, storage method, rate and extent of lignification, and sources. Physical characteristics of foodstuffs such as particle size (PS) has considerable influence on ruminal digestive and metabolic processes. To include information on PS in dietary formulations, the concept of physically effective NDF is receiving increasing attention (Zebeli *et al.* 2012). Regardless of the source, all NDF occupies space in the digestive tract, requires / stimulates chewing activity, and digests slowly and incompletely (Van Soest, 1994). Some characteristics such as digestibility and indigestibility, fragility (Cotanch and Grant, 2008; Zali *et al.* 2015), stem brittleness (Zali *et al.* 2015), and functional specific gravity (Teimouri Yansari *et al.* 2004) may explain the variation in chewing response that's not explained by NDF or peNDF.

The grasses and legumes differ in NDF digestion rates, anatomical structure, and digestion characteristics affecting PS reduction and passage, therefore these differences suggest that consideration of forage family is necessary when evaluating the effects of PS (Kammes *et al.* 2012). Due to variability of an NDF to rumen degradation and the influence thereof on animal performance, knowledge about the digestibility of NDF in forage is critical for effective ruminant feeding. Recently, digestibility and indigestibility of NDF in feeds were considered in their quality assessment and feed digestion models that partitioned the carbohydrates into three fractions of pdNDF, iNDF, and NFC in diets.

Also, Combs (2013) proposed that total tract digestibility of NDF (TTNDFD) is benchmarked to fiber digestibility and can be used in equations to predict forage energy content. As iNDF had no digestibility, is full resistance to the passage and associated to form and consistency of ruminal mat. Therefore, focusing on iNDF and total tract digestible NDF instead of NDF or peNDF alone, in balancing dairy diets leads to take into account the requirement for fiber.

Formulating a diet to a specific level of NDF without reference to the iNDF could markedly affect the resulting intake, digestibility and metabolizable energy (ME) content of the diet (Harper and McNeill, 2015). Therefore, the aim of the present study was to determine indigestible neutral detergent fiber contents of ration ingredients by the *in situ* nylon bag technique and comparison the ruminal degradability of the two rations with similar NDF when their iNDF content are different.

MATERIALS AND METHODS

Experiment 1

For evaluating the effects of indigestible NDF of alfalfa hay and corn silage on rumen degradability with *in situ* method, using four ruminal fistulated Zel ewes (BW=35.5±1.8 kg) that fed a TMR containing 50, 25, and 25% of alfalfa hay, wheat straw, and barley grain and supplemented with mineral/vitamin according to their requirements (CNCPS Sheep, 2007). The animals were adapted to the basal rations for two weeks prior to ruminal incubation of the bags. four grams of dry samples from alfalfa hay, corn silage, barley grain, wheat bran, ration 1, and ration 2 by with four replications were put in nylon bags (6 cm×7.5 cm, porosity 15±2 µ pore size) and incubated in the rumen for 288 h. The composition of the two rations as treatments including concentrates and 1) alfalfa hay, 2) corn silage is indicated in the Table 2, and chemical compositions of the feed ingredients showed in Table 1. The bags were removed after 288 h of incubation (Krizsan *et al.* 2010). Immediately after removing from the rumen, the bags were washed with cold tap water until clear and then were dried at 55 ˚C for 48 h. The bags were weighed and residues were removed and then analyzed.

Feeds and rations were dried at 55 ˚C, ground through a Wiley mill (1-mm screen), and composted by the animal. Samples were analyzed for DM, organic matter (OM), Kjeldahl N, ether extract (EE) (AOAC, 2002), ADF, NDF (Van Soest *et al.* 1991; using heat resistant alpha amylase without sodium sulphate), and ash at 605 ˚C. The non fiber carbohydrates (NFC) was calculated from 100-(CP (%)+NDF (%)+Ash (%)+EE (%)) (Table 1 and 2). The pdNDF was calculated (pdNDF=NDF-iNDF; Raffrenato and Van Amburgh, 2010).

Experiment 2

Using the nylon bag technique, the rations samples were ground through wily mill to pass a 2 mm screen. Then approximately three grams of dry samples were weighed into 7×14 cm^2 and 40 ± 5 µm pore size nylon bags. Bags were incubated in the rumens of three ewes and were removed after 0, 1, 3, 6, 9, 12, 24, 36, 48, 72, 96 h of rumen incubation. Immediately after removing from the rumen, the bags were washed with cold tap water until clear and then were dried at 55 ˚C for 48 h. The bags were weighed and residues were removed and then analyzed for DM, NDF and ADF. The disappearance of DM, NDF at each incubation time was calculated from the proportion remaining in the bag after incubation in the rumen. The disappearance rate was fitted to the following equation given (Ørskov and McDonald, 1979):

$$\text{Disappearance, \%} = a + b \times (1 - e^{-ct})$$

Where:
a: soluble fraction (% of total).
b: degradation fraction (% of total).
t: time of rumen incubation (h).
c: rate of degradation (% h^{-1}).

The effective degradability of DM and NDF was calculated by the equation of Qrskov and McDonald (1979):

$$\text{Effective degradability} = a + [(b \times c) / (c + k)]$$

Where:
k: estimated rate of outflow from the rumen.

Effective degradability of DM and NDF was estimated at ruminal outflow rates of 4, 6, and 8% h^{-1}.

Table 1 Chemical compositions of the feed ingredients

Items	Alfalfa hay	Corn silage	Barley grain	Wheat bran
Dry matter (DM) (%)	86.79	29.29	88.08	89.02
Crude protein (% DM)	14.02	10.79	12.65	18.00
Ash (% DM)	7.85	7.11	2.57	5.29
Ether extract (%)	6.48	9.80	5.70	8.38
Non fiber carbohydrate (% DM)	17.21	15.33	49.54	24.91
Acid detergent fiber (% DM)	44.93	37.14	8.26	15.90
Neutral detergent fiber (% DM)	54.43	57.28	29.48	43.53
$iNDF_{288}$ (% of total)	30.74	16.84	4.77	11.75
$iNDF_{288}$ (% of NDF)	56.50	29.40	16.22	26.99
pdNDF (% of NDF)	43.49	70.59	83.77	73.00

iNDF: indigestible neutral detergent fiber and pdNDF: potential digestible neutral detergent fiber.

Table 2 Chemical compositions of rations and ratio of feed ingredients

Items	Ration 1	Ration 2
Chemical compositions		
Dry matter (DM) (%)	73.38	73.02
Crude protein (% DM)	10.62	11.06
Ash (% DM)	7.53	5.37
Ether extract (% DM)	5.86	3.79
Non fiber carbohydrate (% DM)	23.96	24.09
Acid detergent fiber (% DM)	34.29	27.88
Neutral detergent fiber (% DM)	55.01	55.67
$iNDF_{288}$ (% of total)	21.32	11.42
$iNDF_{288}$ (% of NDF)	41.00	20.52
pdNDF (% of NDF)	58.99	79.47
Ration ingredient (%)		
Alfalfa hay	71.24	-
Corn silage	-	71.66
Barley grain	24.01	15.92
Wheat bran	4.75	12.42

iNDF: indigestible neutral detergent fiber and pdNDF: potential digestible neutral detergent fiber.
Ration 1: for alfalfa hay and concentrate and Ration 2: for corn silage and concentrate.

Statistical analysis

Using a complete randomized design by PROC GLM of SAS (2002), the data in these experiments were analyzed. Separation of treatment means were carried out using the Duncan's multiple range tests with an alpha level of 0.05 and 0.01.

RESULTS AND DISCUSSION

Experiment 1

The chemical compositions of feed ingredients and experimental rations were presented in Table 1 and 2. The feeds had a wide range of chemical composition.

The iNDF content of corn silage, alfalfa hay, barley grain, and wheat bran were 29.40, 56.50, 16.22, and 26.99% of NDF, respectively. The experimental rations were similar in DM, CP, ash, EE, NDF, and NFC content, but the rations were different with $iNDF_{288}$, and pdNDF, and ADF. Zali *et al.* (2015) found that the $iNDF_{240}$ content of wheat bran, corn silage, alfalfa hay, soybean meal, beet pulp, and barley grain were 24.61, 10.23, 22.21, 4.67, 20.48, and 8.86%, respectively.

Cotanch *et al.* (2014) suggested that the determination of iNDF should be included in routine forage and feed analysis because it is a uniform feed fraction with no digestibility. Zali *et al.* (2015) found alfalfa had 22.21% of DM iNDF. Cotanch *et al.* (2014) found that low and high digestibility of alfalfa had 36.7, 7.1, and 15.7; 44.5, 7.5, and, 18.5% of NDF, lignin, and iNDF, respectively. Cotanch *et al.* (2014) found that two type of alfalfa (low and high digestibility) had 36.7, 7.1, and 15.7; 44.5, 7.5, and, 18.5% of NDF, lignin, and iNDF, respectively. Combs (2013) reported that the iNDF fractions in alfalfa and grasses vary from less than 5% to over 55% of NDF, while corn silage iNDF values range from less than 10% to over 40% of NDF (unpublished data). Krizsan *et al.* (2010) reported that iNDF values in a database of 172 feeds ranged from 2.4 to 17.4% of feed dry matter. In this experiment, the $iNDF_{288}$ of corn silage was 16.84% of DM which was higher than 8.4% DM that determined after 288 h ruminal incubation by Huhtanen and Jaakkola (1994). Zali *et al.* (2015) found that $iNDF_{240}$ of corn silage was 10.23%. Although Van Soest (1994) outlined that extent and nature of lignification of forage cell walls control its NDF digestibility, which is a function of various factors, such as forage species, maturity and number of harvests, latitude, and climate.

Experiment 2

Disappearance and effective degradability of DM and NDF of the two rations showed were shown Table 3 and 4, respectively. Dry matter disappearance (in 0 and 2 hours) and NDF disappearance (in 96 hours incubation) in ration 2 were higher (Table 3).The soluble, slowly degraded fractions, and rate of degradation in rations 1 and 2 were 25.93 and 28.93%; 46.26 and 50.40%; 3.19 and 2.36 %/h for DM; 11.74 and 11.81%; 46.32 and 61.37%, and 2.33 and 1.74 %/h for NDF, respectively (Table 4). The ration 2 had greater slowly degraded fraction and degradable fraction of NDF and DM than ration 1. Van Soest *et al.* (2005) showed that the fast pool was exhausted by 48 h, so the choice was made for a time point up to 48 h to represent the fast pool, a point between 48 and 216 h to represent the slow pool, and the 240 h to represent the iNDF fraction. All combinations of the points available (6, 12, 24, 30, 36 and 48 for the fast pool; 72, 96, 120, 144 and 216h for the slow pool) resulted in 30 possible combinations to be optimized. In order to optimize the utilization of forages, fiber digestibility (pdNDF) and the rate at which fiber digests (k_d) must also be accounted for because both greatly affect feed intake and production. Fiber digestibility is more variable than the digestibility of any other feed component and the NDF fraction accounts for 30 to 40% of the digestible energy in high quality forages (Combs, 2013).

Dietary concentration of NDF is related positively to a bulk density of feeds and affects feed intake potential, but its digestibility influences animal performance independent of its concentration (Raffrenato and Van Amburgh, 2010). Volker Linton and Allen (2008) reported that the ruminal NDF digestion rate of an alfalfa based diet and an orchard grass diet were 7.5% and 5.8% per hour, respectively. Corn silages typically have slower fractional rates of NDF degradation than legumes and temperate grasses are more variable in rates of fiber digestion than corn silages or alfalfa (Combs, 2013) which is different from data obtained in this experiment.

Krizsan *et al.* (2010) reported that, the estimated rate of degradation of pdNDF in a database of 172 feeds ranged vary from about 1% per hour to over 10% per hour when measured by using multiple incubation time points and fitting the disappearance of pdNDF to first order kinetics.

Table 3 Dry matter and NDF disappearance of two rations at different incubation times (hours)

Item	0	2	4	6	12	24	36	48	72	96
DM										
Ration 1	23.27^b	26.94^b	31.75	36.69	44.34	54.05	55.58	56.34	68.64	70.01
Ration 2	26.64^a	29.91^a	32.39	37.57	43.11	52.91	56.87	61.00	67.25	73.56
SEM	0.387	0.289	0.565	0.685	0.704	1.786	1.969	1.431	2.075	1.201
P-value	*	**	NS	NS	NS	NS	NS	NS	NS	NS
NDF										
Ration 1	7.01	10.28	15.70	22.86	28.13	34.44	35.97	35.22	50.53	48.10^b
Ration 2	8.44	9.91	15.69	20.48	28.52	37.55	38.90	43.64	49.55	61.56^a
SEM	0.472	0.493	0.458	0.859	1.078	2.147	2.826	2.095	2.929	1.775
P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

Ration 1: for alfalfa hay and concentrate and Ration 2: for corn silage and concentrate.
* (P<0.05) and ** (P<0.01).
NS: non significant.
SEM: standard error of the means.

Table 4 Dry matter and NDF degradability of two rations at different incubation times (hours)

Item	Soluble fraction (%, *a*)	Slowly degraded fraction (%, *b*)	Degradable fraction (%, *a*+*b*)	Rate of degradation (%/h)	Effective degradability in passage rate (%/h)		
					0.04	0.06	0.08
DM							
Ration 1	25.93	46.26^b	72.19^b	3.19	46.45^b	42.00^b	39.12^b
Ration 2	28.93	50.40^a	79.34^a	2.36	47.63^a	43.16^a	40.41^a
SEM	0.863	0.888	0.646	0.273	0.599	0.578	0.557
P-value	NS	*	**	NS	**	**	**
NDF							
Ration 1	11.74	46.32^b	58.07^b	2.33	28.79^b	24.70^b	22.19^b
Ration 2	11.81	61.37^a	73.19^a	1.74	30.41^a	25.60^a	22.77^a
SEM	1.034	1.089	1.195	0.329	1.070	1.013	0.960
P-value	NS	***	***	NS	***	***	***

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
Ration 1: for alfalfa hay and concentrate and Ration 2: for corn silage and concentrate.
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.
SEM: standard error of the means.

A faster disappearance of the NDF fraction from the rumen because of the increased rate of digestion or passage might reduce physical fill in the rumen over time and allow greater voluntary feed intake. Forage digestibility is constrained by the iNDF and the rate of digestion of pdNDF.

Fiber and lignin increase while digestion rates decline with forage maturity. It is well recognized that lignin content is a key intrinsic factor that limits cell wall digestibility in forages (Dann *et al.* 2006) and affects iNDF content of forages. The iNDF has been characterized as the most important factor affecting the digestibility of the total diet OM (Nousiainen *et al.* 2004). Estimates of the slow pool of NDF, its rate of digestion, and undigested NDF (uNDF) are related to dry matter intake and the passage from the rumen.

The uNDF plays a critical role in maintaining the ruminal digesta load, and predicts forage quality because of the relationship between uNDF and OM digestibility (Cotanch *et al.* 2014).

CONCLUSION

Neutral detergent fiber digestibility is an important factor for predicting energy content of forages in nutritional models. Fermentation curves of NDF can be partitioned into three components: a fast digesting pool, a slow digesting pool, and an indigestible fraction. The rate of digestion k_d is an average of fast and slow digesting pools and is valid up to 36 hours, after which the fast pool has decayed out and rate of digestion at 48 hours and later is dominated by the slow second pool (a point between 48 and 216 h). Forage digestibility in ruminants is constrained by the extent of cell wall (NDF) digestion. Incomplete degradation of cell walls is a major factor limiting the value of forages to animals. In this experiment, the iNDF content in alfalfa hay was higher than corn silage; dry matter and NDF disappearance and degradability in ration 2 (corn silage content) were higher than ration 1 (alfalfa hay content). This experiment results showed that non-uniform of the NDF fraction of rations with similar chemical composition, can affect ruminal degradability of dry matter and NDF.

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