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FORAGE PROTEIN FRACTION VALUES DETERMINED BY AN *IN SITU* METHOD (NDIN PROCEDURE)

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Story in Brief

A modified neutral detergent fiber insoluble nitrogen (NDIN) procedure was used to measure degradable (DIP) and undegradable (UIP) protein fractions of forages. The NDIN procedure estimates the amount of UIP associated with feeds by determining the extent and rate of disappearance of fiber-bound nitrogen (NDIN). This procedure allows for determination of forage UIP using a combination of previously standardized procedures. Estimates of forage UIP, as well as fiber digestibility are important measurements necessary for accurate predictions of cattle performance based on the current NRC equations. Three trials with varied forage types were conducted using this procedure to determine protein fractions in order to develop feeding recommendations according to NRC (1996) guidelines. Forage quality of Plains Old World bluestem and tallgrass prairie changed with advances in the growing season. Protein fractions also changed with season. Supplementation of prairie hay altered protein fractions. These results illustrate the need to be aware of seasonal effects and changes in ruminal digestibility due to supplementation when evaluating rations for DIP using NRC (1996) software.

Key Words: Forage Protein Fractions, Ruminal Degradability, NDIN

Introduction

For nutritionists and producers to develop feeding programs using the metabolizable protein (MP) system proposed by the NRC (1996), ruminal degradation of forage protein must be estimated. The MP system divides protein into a ruminally degradable fraction (Degradable Intake Protein; DIP) and a ruminally undegradable fraction (Undegradable Intake Protein; UIP). Effectiveness of the MP system depends on accurate estimates of these fractions to predict animal performance from MP available to the animal.

Research conducted at the University of Nebraska (Mass et al., 1999) indicated that neutral detergent fiber insoluble nitrogen (NDIN) could be used to estimate forage UIP. In initial trials, UIP estimates calculated from NDIN results were closely correlated (R^2=.954) with *in vivo* estimates of UIP. The most recent NDIN procedure (Mass et al., 1999) is a rapid procedure to determine forage UIP values while only allowing for determination of NDIN. Due to a small initial sample size, important additional determinations of dry matter and fiber disappearance cannot be estimated. The NDIN procedure performed at Oklahoma State University follows traditional *in situ* and NDF procedures more closely, while allowing multiple measurements to be made. This procedure will allow the inclusion of these measurements in a forage quality calendar for Oklahoma.

Materials and Methods

Animals and Diets. Three trials were conducted with three forage types in 1997 and 1998. Forages (Table 1) included masticate samples from dormant native range (tallgrass prairie (TGP); Vermeulen, 1998), growing season Plains Old World bluestem (OWB) and baled prairie hay (PRAH). Forage samples were incubated *in situ* using ruminally cannulated cattle adapted to diets for 10 d. Forage intakes ranged from 1.5 to 2.0% of BW (DM) with free choice access to clean water and mineral salt mix balanced to meet animal

requirements (NRC, 1984). Since original equations were obtained by feeding brome hay (8 to 10% CP, fed at 1.8% BW), hay similar in digestibility and TDN would be preferred. However, in trials at OSU either prairie hay (PRAH; 5.3% CP) with supplements or mixed cool-season perennial hay (CSPH; 18.5% CP) was fed. To maintain dietary CP levels of at least 7% (to avoid nitrogen deficient diets) with PRAH, supplements were fed. Supplements included HPROT, 3 lb/hd/d soybean meal (47% CP) or one of four monensin-containing (200 mg/hd/d) supplements consisting of: 1) FIBER (wheat midds/soybean hulls) or 2) GRAIN (milo) 15% CP energy supplements fed at 1% BW, 3) MPROT (cottonseed meal/distillers grain-based) 25% CP supplement fed at 0.5% BW and MIN (trace mineral salt mix) fed at 4 oz/hd/d.

Incubations/Rinsing. Duplicate 10 x 20 cm dacron bags were prepared for each incubation time (2, 12 and 96 h) for each animal. Bags were dried at 50° C for 24 h and allowed to equilibrate to atmospheric moisture prior to weighing. All weights were obtained on an air-dry basis because the hygroscopicity of the polyester bags compromised the ability to obtain accurate moisture-free weights. Forage samples were dried, ground (2 mm screen) and sub-sampled for DM and OM determination. Dacron bags were filled with 5g (air equilibrated ~ 90% DM) of forage creating a ratio of 12.5 mg of sample/cm^2 of bag surface area. Bags were heat-sealed in two locations and care was taken to not overly decrease the surface area of the bag by sealing too far down. No more than 20 dacron bags were placed into 36 x 42 cm polyester mesh bags fitted with nylon zippers and no more than 120 dacron bags were placed in the rumen of any animal. Prior to incubation in the rumen, bags were soaked in 39° C water for 20 min. Bags were inserted in reverse order of incubation time at -96 h, -12 h, -2 h with all bags removed at time 0. Bags were rinsed with tap water to remove particles (in Trials 1 and 3 bags were frozen prior to washing) and washed using a washing machine by manually manipulating the rinse and spin cycles as follows: Machine set to gentle agitation, low water level, cold water temperature, filled with water and allowed 1-min rinse and 2-min spin cycles. This procedure was repeated nine times for a total of 10 complete cycles. Bags were dried (50° C) for 24 h and re-weighed. Again, bags were allowed to air-equilibrate prior to weighing. Sub-samples (normally 0.5 g) were taken from residue remaining for NDF, N and DM determination. NDF values were calculated on an air-equilibrated basis and recorded for later use. The post-NDF residue was thoroughly rinsed, dried, air-equilibrated, weighed and recorded. Post NDF residue was analyzed for N and expressed as a percentage of the air-equilibrated weight of the post NDF residue.

Statistical Analysis. Forage NDF, CP and UIP as a percentage of DM (%DM) were regressed on month using the REG procedure of SAS (1996) to determine linear trends over time. Correlations of the same variables and months were tested using the CORR procedure of SAS (1996).

Calculations. Calculations and required factors used to determine forage UIP values from the *in situ* NDIN procedure are listed. The basis of these equations is that passage rate (Kp) and rate of digestion (Kd) interact to determine extent of digestion. Incubated forage Kp values were used as determined by marker dilution techniques or from previously published values for a similar diet. Kd was calculated by determining the amount of NDIN remaining at 2 and 12 h corrected for ruminally undegraded NDIN (NDIN remaining after 96 h incubation), converting these values to first order kinetics using natural logarithms and determining the slope (Kd) of the line connecting the two points (2 and 12 h). These values are a function of first order kinetics and are expressed in percent per hour. The equation also requires the use of an "original pool" of NDIN. This can be thought of as the fiber-bound N in the original sample of forage and could be measured by determining the NDIN of the forage. The preferred method that corrects for different rates of digestion (Kd) is to use the inverse natural log of the y-intercept value from the Kd equation as the "original pool" of NDIN. Kd, Kp, and "original pool" of NDIN are used to calculate UIP as %DM using Equation 1. The following values and descriptions outline the steps used to perform

this procedure and what each value represents.

VALUES DESCRIPTION

A) g of original sample air-equilibrated grams of forage prior to ruminal incubation

B) g of sample remaining air-equilibrated grams of forage remaining after 2, 12 or 96 h of ruminal incubation

C) % NDF % NDF of post-ruminal incubated residue determined on air-equilibrated basis

D) %N in NDF % N of NDF residue determined on air-equilibrated basis from residue remaining post NDF

E) g of NDF (B*C) grams of NDF post ruminal incubation, calculated by multiplying **B** (g of sample left) by **C** (%NDF)

F) g of NDIN (D*E) grams of NDIN post ruminal incubation, calculated by multiplying **D** (%N in NDF) by **E** (g of NDF)

G) g of NDIN/g (F/A) grams of NDIN post ruminal incubation expressed as a proportion of the original sample, calculated by dividing **F** (g of NDIN) by **A** (g of original sample)

H) mg of NDIN/g mg of NDIN per gram of original sample, calculated by multiplying **G** by 1000

I) Corrected mg NDIN/g mg of NDIN/g for 2 and 12 h corrected by subtracting the NDIN remaining after 96 h of ruminal incubation. This is calculated by subtracting **H** (mg of NDIN/g at 96 h) from **H** (mg of NDIN/g at 2 and 12 h)

J) ln mg NDIN/g natural logarithm of corrected mg NDIN/g for 2 and 12 h, calculated by taking ln of **I**

K) rate of digestion (Kd) rate of digestion of corrected NDIN as determined by the absolute value of the transformed data□s slope: 2 h: ln(2.9321) = 1.0757

12 h: ln(2.4339) = .8895

Kd = (1.0757 - .8895)/(2-12) = -.0186 = 1.86%/h

L) y-intercept y-intercept value from rate of digestion regression equation, calculated as:

Y=1.0757+(-.0186)*(-2)=1.113

M) "original NDIN pool" "original sample" mg NDIN/g, calculated by taking the inverse

natural log of **L** (y-intercept)

$e^{1.113} = 3.0435$

N) rate of passage (Kp) rate of passage of digesta through the rumen. This must be determined using marker dilution techniques or obtained from previous estimates

O) extent of digestion amount digested, calculated by dividing **N** (Kp) by **N**+**K** (Kp + Kd)

P) UIP as %DM of forage percentage of forage DM undegradable intake protein (UIP), calculated by multiplying **O** (extent of digestion) by **M** ("original pool"). The product is then divided by 1000 (converting mg to g), multiplied by 6.25 (to convert N to CP) and 100 (to convert from decimal to percent)

Q) UIP as %CP of forage UIP as a % of forage DM can be converted to UIP as a % of CP by dividing **P** (UIP as % of forage DM) by %CP of original forage

R) DIP as % of forage CP Determined by subtracting **Q** (UIP % CP from 100)

Results and Discussion

Forage quality (NDF, %CP) and protein fractions (expressed as UIP and DIP as %DM) are listed in Table 1. Advancing season caused changes in NDF, CP, UIP and DIP (%DM) of forages while supplementation did not affect forage NDF or CP, but did alter DIP and UIP (%DM). Values for DIP and UIP as a percentage of CP (%CP) are not reported but can be calculated from Table 1 by dividing UIP or DIP (%DM) by CP concentration (DM).

Old World Bluestem. A linear decrease ($P<.02$, $R^2=.96$) in CP and DIP %DM ($P<.07$, $R^2=.86$) was noted as the growing season progressed into late summer. However, no difference as growing season advanced was observed in UIP ($P>.20$, $R^2=.63$) values when expressed as %DM for OWB. This indicates that as the growing season progresses, changes in forage quality can increase UIP %CP resulting in decreased DIP %CP.

Tallgrass Prairie. A linear decrease ($P<.06$, $R^2=.89$) in NDF concentration of tallgrass prairie forage was found as growing season progressed into early spring. A tendency for a linear increase ($P>.18$, $R^2=.67$) was noted for UIP %DM of TGP during late winter and early spring. While CP was highly correlated (r=.77) with each advance in month, these increases in CP are composed of more UIP relative to DIP %DM. This agrees with our findings of a linear decrease ($P<.14$, $R^2=.74$) in DIP %CP values.

Supplementation of Prairie Hay. Feeding prairie hay with four supplements resulted in changes in UIP and DIP while the concentration of NDF and CP remained constant. Supplementation affected passage rate, rumen retention time, intake and digestibility of forage. Changes in these factors combine to alter the amount of forage CP that is ruminally degraded. However, increased intake of forage will increase CP intake and consequently increase absolute DIP intake or supply to the rumen, even though DIP concentration (% DM) was decreased in the hay.

Conclusions

Seasonal and(or) yearly changes in levels of CP, UIP or DIP in forages due to climate, maturity or diet selection will alter DIP supplied to rumen micro-organisms and

consequently affect MP status of cattle grazed or fed these forages. Altering ruminal fiber degradation by supplementation will decrease available DIP concentration in the forage and consequently affect MP supply to ruminant livestock fed low quality prairie hay forage. However, supplementation may increase intake of forage and also increase DIP intake causing an increase in absolute quantity of DIP reaching the rumen. This increase in DIP intake may overcome the decrease in DIP concentration and provide higher MP supply improving animal performance. Supplement source must also be carefully considered since this will alter rumen kinetics differently. Supplements containing high protein levels from oilseed meals are typically thought to increase passage, while supplements high in starch and low in protein are commonly believed to decrease both digestion and intake.

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Equation 1. Calculations used to express UIP as %DM of forage (P).

O	*	**M**	÷	1000 (mg to g)	*	6.25 (N to prot)	*	100 (convert to percent)	=	**P** (UIP, % of Forage DM)

Table 1. Forage neutral detergent fiber, crude, degradable and undegradble protein fractions for three Oklahoma forages.

Forage type	NDF	CP	UIP %DM[1]	DIP %DM[1]
Old World bluestem[2]				

June	82.50	13.44	2.56	10.88
July	80.14	12.95	2.18	10.77
August	80.40	11.61	2.45	9.16
September	79.50	10.11	1.10	9.00
Tallgrass prairie[3]				
February	69.90	7.80	2.30	5.50
March	68.40	5.40	1.50	3.90
April	61.90	14.00	6.60	7.40
May	49.50	13.20	6.10	7.10
Prairie hay[4] *fed with:*				
Fiber supplement[5]	72.60	5.49	1.69	4.25
Grain supplement[6]	72.60	5.49	1.81	4.13
Protein supplement[7]	72.60	5.49	1.36	4.58
Trace Mineral Mix[8]	72.60	5.49	1.21	4.73

[1]See sample calculations.

[2]*Bothriochloa ishaemum* (var. Plains) masticate samples.

[3]Dormant native winter range masticate samples (Vermeulen, 1998).

[4]Tallgrass prairie hay fed with four monensin-containing (200mg/hd/d) supplements.

[5]Wheat midds/soybean hulls-based 15% CP fed at 1% of BW.

[6]Sorghum grain-based 15% CP fed at 1% of BW.

[7]Cottonseed meal/distillers grain-based 25% CP fed at 0.5% of BW.

[8]Trace mineral salt mix fed at 4 oz/hd/d.

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