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TEST OF A CONVERSION EQUATION TO INCREASE THE ACCURACY OF MICROHISTOLOGICAL ANALYSIS OF HERBIVORE DIETS IN THE PANTANAL

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Abstract

Forty forage species samples were collected in September 1999, including grass, sedges, forbs, shrubs and trees. Seven hand-compounded mixtures of known botanical composition and dry weight were prepared. Five slides of each mixture were made and 20 frequency observations were recorded per slide. Two procedures were used to determine percent composition on a dry weight basis and their values were converted to correct the proportion of the unidentifiable fragments amongst species. Estimated and actual values differed significantly ($P<0.05$) in 31.7%, 34.1%, 12.2% and 12.2% of the species for frequency addition (FA), density convertion (DC), frequency addition converted (FAC) and density convertion converted (DCC), respectively. The average similarity values between estimated and observed mixture were 90.2, 86.0, 94.2 and 93.3 for FA, DC, FAC and DCC, respectively. In conclusion, food habits by microscopic analysis may be described using any of the procedures tested because they rank important forage species for herbivores.

Keywords: Native pastures, botanical composition.

Introduction

The fecal microhistological technique based on the frequency of occurrence of plant fragments on a microscope field (presence or absence) has become one of the most popular methods of determining food habits of large herbivores (Mcinnis *et al*., 1983). However, this method presents several limitations. Several studies have evaluated its accuracy (Sparks and Malechek, 1968; Holechek and Gross, 1982; Mcinnis *et al*., 1983; Norbury, 1988) . Known mixtures may be used by all technicians to evaluate their accuracy and provide factors for correction if certain species are over or under estimated (Holechek and Gross, 1982). One of the limitations refers to the fact that the relation between identifiable epidermal tissue and unidentifiable tissue is not similar for all species, overestimating or underestimating some of them. Norbury (1988) elaborated a conversion equation based on the proportion of identifiable tissues for each species. This equation may provide an accurate analysis of herbivore diets.

The objective of this study is to evaluate the use of this conversion equation for microhistological analysis of herbivore diets in the Pantanal. For this purpose two procedures are used for calculating the dry weight composition: addition frequency and frequency converted to density.

Material and Methods

Forty species were selected for experimental use, based on preference and abundance. Cattle preferred parts were harvested and kept in a refrigerator until processing. Species used in the diets included grasses: *Axonopus purpusii*, *Panicum laxum*, *Paspalidium paludivagum*, *Leersia hexandra*, *Hymenachne amplexicaulis*, *Reimarochloa*

brasiliensis, Paspalum plicatulum, Andropogon hypogynus, Mesosetum chaseae, Andropogon bicornis, Sorghastrum setosum, Elyonurus muticus, Andropogon selloanus, Setaria geniculata, Axonopus paraguayensis and *Loudetia flammida; sedges: Eleocharis acutangula, Rhyncospora trispicata, Rhyncospora tenuis, Cyperus haspan, Eleocharis minima* and *Eleocharis interstincta;* forbs: *Diodia kuntzei, Richardia grandiflora, Psittacanthus calyculatus, Sebastiana hispida, Bidens gardneri, Thalia geniculata, Melochia simplex, Aeschnomone fluminensis, Hydrolea spinosa, Desmodium barbatum* and *Hyptis brevipes* ; shrubs: *Annona dioica, Smilax fluminensis, Arrabidea brachypoda, Doliocarpus dentatus* and *Banisteriopsis pubipetala;* trees: *Curatella americana* and *Scheelea phalerata.*

Seven mixtures were hand-compounded to represent simulated diets. Five slides of each species and each mixture were prepared based on the methodology of Sparks and Malechek (1968). Only one observer analyzed both mixture and reference slides using Nikon binocular microscopes at 100 magnification. Drawings, photographs, microscope slides and keys were developed for separating these species on the basis of epidermal and celular characteristics. Twenty frequency observations were recorded per slide to insure high repeatability among slides. Two procedures were used to calculate the percentage of dry weight. In the first procedure the number of identifiable fragments of each species in all fields was divided by the total number of identifiable fragments of all species in all fields (Holechek and Gross, 1982). Procedure two involved the conversion of frequency to density described by Fracker and Brischle (1944).

The proportion of identifiable epidermal tissue was determined for each species by examining the reference slides of chopped samples of individual species. A convertion equation was calculated as discussed by Norbury (1988) for both procedures:

$$\text{Converted point frequency} = [fi/f(IDi)]/ \sum_{i=1}^{n} [fi * f(IDi)]$$

Where fi = point frequency of epidermis for species i in the mixture, f (IDi) = point frequency of identifiable epidermis for species i on the reference slide, and n = total number of species in the mixture.

Similarity indices were calculated using Kulcyznski's formula (Oosting, 1956) to show the similarity between estimated and actual dry weight composition. The estimated percentage of each species in each mixture was compared to the actual percentage using a t-test for paired comparisons.

Results and Discussion

Estimated and actual values differed significantly ($P<0.05$) in 31.7 %, 34.1%, 12.2% and 12.2% of the species for frequency addition, density conversion, frequency addition converted and density conversion converted, respectively (Table 1). The similarity values between estimated and observed diets for the two procedures and their converted point frequencies suggest that the test of accuracy applied to the two procedures increased the estimated value, especially for the density conversion procedure (Table 2). Without the use of converted point frequency, the frequency addition procedure provided the most accurate evaluation of dry weight composition for almost all mixtures. Similar results were found by Holechek and Gross (1982) and Alipayo *et al.* (1992). Barker (1986) suggests that, to make the use of correction factors worth, the accuracy should be at least doubled. According to Holechek and Gross (1982) the frequency addition procedure reduces overestimation of species with easily identifiable fragments while the density procedure either magnifies or reduces it depending on whether the frequency of the overestimated

species is low or high in relation to other species in the mixture. These cases were also observed in this study as in *A. purpussi* (mixture 1), in *H. amplexicaulis* (mixture 2), *P. plicatulum* (mixture 3) and *D. dentatus* (mixture 7). These species constitute major mixture components. The species estimated values were reduced by frequency addition and magnified by density procedure. However, the use of the test of conversion increased these values. The results indicated that food habits by microscopic analysis of fecal material may be described using any of the procedures tested because they rank forage species important for herbivores. Based on these data, the frequency addition procedure with converted values gives better accuracy for calculating the percentage of dry weight. However, as the use of correction factors did not greatly improve the accuracy, its application could not be justified.

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Table 1- The percentage mean (±sd) of dry weight of seven hand-compounded mixtures, using two methods (fia and fib) and converted frequency.

Mixtures	Actual % dry weight	% dry weight using frequency (fia)	% dry weight using frequency to density (fib)	F (IDi)[1] reference slide (%)	Converted point frequency using fia	Converted point frequency using fib
Mixture 1						
A. purpusii	41.8	39.5 ± 1.5*	50.1 ± 4.1*	86.0	36.3 ± 1.6*	46.8 ± 4.5
P. laxum	21.2	23.4 ± 2.8	20.7 ± 3.1	81.0	23.4 ± 3.5	20.3 ± 3.1
E. acutangula	20.9	18.3 ± 4.6	15.3 ± 5.7	70.0	20.5 ± 5.3	17.5 ± 6.2
L. hexandra	8.5	11.5 ± 2.8	8.6 ± 2.4	87.0	10.4 ±2.5	7.8 ± 1.5
P. paludivagum	7.6	7.3 ± 1.1	5.1 ± 0.9*	56.0	10.0 ± 1.5*	8.2 ± 1.5
Mixture 2						
H. amplexicaulis	26.8	31.2 ± 4.1*	44.8 ± 7.7*	84.0	27.8 ± 4.1	33.4 ± 6.8
D. kuntzei	26.8	22.7 ± 3.5*	21.5 ± 3.4	45.0	28.2 ± 2.7	27.8 ± 3.8
Reimarochloa sp	20.3	20.6 ± 4.4	20.1 ± 5.4	55.0	21.6 ± 4.8	21.3 ± 5.8
R. tenuis	13.8	13.6 ± 3.6	12.3 ± 3.7	62.0	12.9 ± 3.4	11.7 ± 3.6
R. trispicata	12.3	12.5 ± 3.3	11.2 ± 3.8	57.0	12.6 ± 8.0	11.5 ± 3.6
Mixture 3						
P. plicatulum	27.5	38.5 ± 4.1*	44.8 ± 7.7*	84.0	27.8 ± 4.1	33.4 ± 6.8
A. hypogynus	22.3	15.1 ± 3.7*	13.1 ± 3.1	44.0	20.6 ± 3.8	18.8 ± 3.9
M. chaseae	22.0	20.8 ± 5.2	19.6 ± 5.3	67.0	18.6 ± 3.5	18.3 ± 4.7
A. bicornis	12.3	8.3 ± 1.8*	7.4 ± 2.1*	37.0	13.6 ± 2.5	12.5 ± 3.4
C. haspan	10.7	7.6 ± 1.7*	6.1 ±1.7	40.0	11.4 ± 2.7	9.6 ± 2.8
R. grandiflora	5.2	10.3 ± 2.6*	8.7 ± 2.8*	74.0	8.4 ± 2.5*	7.4 ± 2.2*
Mixture 4						
S. setosum	24.4	15.5±4.7*	15.0±5.8*	50.0	19.9±4.8	19.3±6.0
E. muticus	19.9	19.0±3.4	20.2±6.5	80.0	15.7±3.5	19.1±3.8
A. selloanus	11.7	9.4±2.5	7.8±2.1	42.0	14.4±3.1	12.2±2.7
A. dioica	10.6	15.5±5.7	15.1±6.1	90.0	11.1±4.1	11.3±5.3
C. americana	9.6	16.8±1.3*	16.4±1.0*	85.0	12.3±1.3*	13.4±1.2*
S. hispida	9.0	5.7±1.6*	4.7±1.7*	56.0	7.6±2.9	5.6±1.9*
P. calyculatus	8.8	12.9±4.2	11.8±4.7	82.0	10.1±3.8	10.9±3.7
B. gardneri	6.0	7.9±2.9	6.5±2.6	51.0	9.8±3.0	8.2±2.6
Mixture 5						
T. geniculata	22.8	22.5±3.2	25.9±5.0	75.0	18.0±2.9*	21.0±4.3
M. simplex	21.5	16.5±4.4	17.1±6.0	53.0	18.7±4.6	19.3±6.2
E. minima	11.1	14.0±1.8*	13.8±2.1*	80.0	10.5±1.6	10.6±1.8
A. fluminensis	11.0	7.0±1.4*	6.1±1.4*	40.0	10.5±2.1	9.3±2.0
R. trispicata	9.8	12.4±1.0*	11.8±1.2*	57.0	13.0±0.8*	12.4±4.0*
E. interstincta	8.5	8.9±2.7	8.1±2.8	40.0	13.2±3.9	12.2±4.0
S. geniculata	8.1	10.9±3.5	10.3±4.0	82.0	7.8±2.8	7.7±3.2
H. spinosa	7.2	7.8±2.4	6.9±2.3	56.0	8.1±2.7	7.5±2.6
Mixture 6						
D. barbatum	23.5	25.2±2.7	26.0±4.4	69.0	23.9±2.6	25.6±4.0
S. phalerata	22.1	28.9±5.6*	32.1±9.1*	95.0	20.2±4.1	23.0±7.1
A. paraguayiensis	20.5	8.8±1.4*	7.0±1.1*	30.0	19.5±2.8	16.0±2.4*
S. fluminensis	17.7	20.1±4.3	19.2±5.6	71.0	18.7±4.0	18.6±5.4
A. brachypoda	16.2	16.9±4.9	15.5±6.1	62.0	17.7±4.9	16.8±6.0
Mixture 7						
D. dentatus	41.4	45.8±1.5*	60.7±2.5*	85.0	36.9±1.8*	51.6±2.7*
B. pubipetala	25.0	20.9±3.7*	15.8±4.0*	55.0	26.1±4.5	20.7±5.0
L. flammida	20.7	17.7±4.0	12.8±3.3*	55.0	22.1±4.6	16.8±4.0*
H. brevipes	12.9	16.0±3.8	11.3±3.0	75.0	14.8±3.5	10.9±3.2

1- f(IDi) = point frequency of identifiable epidermis for species i on the reference slide

* Observed value differs significantly ($P<0.05$) from the actual value, by paired t-test

Table 2 - The similarity index between actual and estimated mixture mean percentage in two procedures (frequency addition and frequency converted to density) and their converted point frequencies.

Mixtures	Frequency addition (fia)	Frequency converted to density (fib)	Fia converted	Fib converted
1	94.8	91.4	94.1	95.0
2	95.7	91.9	97.0	97.1
3	84.5	79.1	94.9	91.7
4	84.6	82.4	89.9	90.7
5	90.7	90.0	91.0	93.4
6	88.3	85.8	97.1	95.5
7	92.9	81.3	95.5	89.8
Mean	**90.2**	**86.0**	**94.2**	**93.3**