ASSESSMENT OF MICROHISTOLOGICAL TECHNIQUES FOR DETERMINING DIET OF GREATER ONE-HORNED RHINOCEROS (*RHINOCEROS UNICORNIS*)

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ABSTRACT

I collected fresh fecal samples of greater one-horned rhinoceros *Rhinoceros unicornis* from Nepal's Royal Chitwan National Park to assess the microhistological technique for determining diet. The microfecal analysis based on the line intercept method provides satisfactory estimation of the range of plant species and their volumetric contribution in the diet. Over 90% of the plant species were identified. Volumetric contribution of plants that are moderately and less preferred is sensitive to size of sample and number of slide transects. To estimate 90% of the volume contributed by these species, samples from more than eight different animals and the readings of a minimum of 20 transects/animal are required. Slide preparation and reading of individual samples of the line-intercept method is laborious. Estimating the volumetric contribution of species by the frequency distribution of fragments encountered is less laborious and give similar results as measuring size of each fragment. Also, pooling samples from different animals reduces the time required for analyzing individual samples with little loss of precision.

INTRODUCTION

Methods for studying diet of free-ranging wild herbivores are direct field observation, feeding trials, clipping and browsing studies and microhistological techniques. Among these, microhistological techniques for examining esophageal (McInnis et al. 1983, Elliot and Barrett, 1985, Kirby and Parman 1986), rumen (Bergerud and Russell 1964, Mitchell and Smoliak 1971, Branan et al. 1985, Lewis 1994), and fecal samples (Stewart 1967, Kessler et al. 1981, Migongo-Bake and Hansen 1987, Alipayo et al. 1992) are most favored.

Limitations associated with esophageal fistula include contamination by rumen contents, incomplete recoveries of fistulated animals, high cost and low precision in sampling for individual species (Holechek et al. 1982). Collection of esophageal and rumen samples also requires sacrificing several animals, which is not feasible when studying rare and endangered species.

In recent years fecal analysis has been found to be a reliable method for estimating the composition of diet of grazing herbivores (Todd and Hansen 1973, Johnson and Pearson 1981, Larter and Gates 1991, Alipayo et al. 1992). However, differential digestion may seriously affect precision of the microhistological analysis among ruminants (Slater and Jones 1971, Anthony and Smith 1974, Fitzgerald and Waddington 1979, Smith and Shandruk 1979, McInnis et al. 1983, Holechek and Valdez 1985, Vavra et al. 1978), but this has been questioned by Alipayo et al. (1992). Such limitations do not apply to the same extent to monogastric species like rhinoceros.

The microhistological technique is based on enumerating identifiable fragments in a certain number of microscopic fields (Sparks and Malechek 1968) or on the line-intercept method (Seber and Pemberton 1979). A main drawback of the technique is that it is time

consuming.

The present study is based on analysis of fecal samples from a free-ranging population of wild greater one-horned rhinoceros (*Rhinoceros unicornis*, henceforth termed rhino) in Royal Chitwan National Park (RCNP), Nepal. The main purpose of the study was to determine the sample size (number of slide transects and number of animals) required to identify the range of food plants and to estimate the relative volumetric contribution of the food plants in the diet of rhinos.

METHODS

Types of fecal sample

I collected two sets of fecal samples from RCNP within an area of approximately 2 km^2 of riverine forest and adjacent floodplain grasslands along the Rapti river near Sauraha. The first set was collected from 10 known animals and the second from 20 unknown animals. Samples were collected over a 3 day period during the monsoon in September 1993. The set of 20 unknowns were pooled into groups of five, ten, fifteen and twenty. The purpose was to assess intraspecific variation and to determine sample size needed for adequate representation of food plants and their relative proportion in the diet.

Preparation of fecal sample

Each fresh dung pile, defecated at one time, was thoroughly mixed. Approximately 400 g (wet weight) was extracted, air dried, ground to pass through a 1 mm screen and sieved through a 210 mu micron Endcott sieve to ensure homogenous size of the fragments and to

remove dust and fine unidentifiable particles.

About one tablespoonful of ground dung was transferred into test tubes to which warm 5% sodium hydroxide (NaOH) solution was addeded. The test tubes were heated in a boiling water bath for 4-6 minutes and allowed to cool. The supernatant dark fluid in the test tubes was decanted, replaced with fresh NaOH, and repeated 2-3 times until a clear solution was produced. The material was then washed with warm fresh water and absolute alcohol to eliminate the NaOH. Finally, the sample was dehydrated through a series of alcohol and xylene (75, 50, 25 percent) mixtures (Anthony and Smith, 1974).

A small amount (equal in all slides) of dehydrated material was uniformly mounted in canada balsam under a 24 X 50 mm cover slip. Five slides were prepared for each dung sample. The slides were air dried for 5-7 days before analysis.

Zyznar and Urness (1969) used NaOH to clean deer feces and reported low percentage of discernable fragments. Their procedure of treating fecal pellets with NaOH might have influenced the identifiable characteristics of the fragments. They either soaked the fecal pellets over night or boiled them for 15 minutes in 10% NaOH and later stirred vigorously to reduce the material into a pulpy mass. Direct boiling in NaOH and vigorous stirring results in disintegration of fragments. Anthony (1972) also found boiling time to be critical for microfecal analysis.

Procedure for reading slides

Identification of each plant species was based on epidermal characteristics as described by Spark and Malechek (1968) and Storr (1961). The line-intercept method (Seber and Pemberton, 1979) was employed to estimate the proportion of different plant species. Five horizontal transect-lines were randomly located on each slide by moving the slides with a

rotating attachment on the microscope. The length of all fragments intercepted by the line was measured to the nearest 0.04 mm with a calibrated ocular micrometer. Each transect was examined under 200X magnification or 500X magnification.

Reference slides used in this study was available from an ongoing vegetation study (Jnawali, in prep.). Above ground parts (leaves, twigs, fruits and flowers) of over 100 different plant species collected from the study area were shredded coarsely using an ordinary electric blender. A teaspoonful of the coarse material was transferred separately into test tubes, marked and mixed with 10% NaOH to clean the epidermal tissues. Dehydrating and mounting procedures were the same as for the fecal material.

RESULTS AND DISCUSSION

General

A total of 28 species of plants (15 grasses, 7 browse and 6 others, including sedges, herbs, ferns and horsetails) were identified from the 10 known but different animals. Mean composition of each species is given in Table 1. Grass species composed about 65%, browse species 20%, and others less than 5%. Unidentifiable fragments averaged 6% of the total volume.

From direct feeding observations Laurie (1978) recorded over 100 species of plant eaten by rhinos in RCNP. However, his results were based on the entire year of a larger area, a wide variety of agricultural crops and associated species, and aquatic plants. Rice, the only available crop during the time of sample collection, was in a very early stage of growth. Similarly, access to aquatic species was restricted because of high flooding during the

monsoon 1993, and aquatic plants are eaten mostly during other times of the year (Laurie 1982).

In ruminants investigators report difficulties in identifying species of forbs because they are more thoroughly digested and, as a result, are under-estimated (Storr 1961, Free et al. 1970, Pulliam and Nelson 1979, Vavra and Holechek 1980). Due to low assimilation, fragments of forbs in the present sample were identifiable, and their percent composition was low, all < 1%.

Westby et al. (1976) reported that woody remnants possess less discernable characters than grass species in fecal material. Holechek and Valdez (1985) also reported that fecal analysis underestimates species of shrubs high in stemmy material. Here grass-species constituted about 65% of total volume. Among browse species, the highest contribution was calculated for *Trewia nudiflora* (13.4%), consisting only of fruity parts with discernable features. The influence of woody remnants would have been expected when stems of browse species dominate the diet, particularly in shortage of palatable species of grass during the dry season. However, during the monsoon rhinos eat only the fruity parts of *Trewia nudiflora* (Dinerstein and Wemmer 1988).

Coefficients of variation (CV) were calculated for all plant species detected. Variation was low for all three key species, *Trewia nudiflora* (17.9%), *Saccharum spontaneum* (24.6%), and *Narenga porphyrocoma* (30%); while variation was noticeably higher among moderately and less preferred species (Table 1). The CV decreased significantly ($R^2 = 38.6$, $p < 0.001$) with increasing relative proportion of plant species in the diet.

Analysis of individual dung samples

Among 10 different animals, three were randomly selected to determine the number of transects needed to record the range of plant species in the diet. An average number of 15 transects were required to record at least 90% of the plant species present (Fig. 1). Hence, with five transects on each slide, a minimum of four slides were needed for this level of precision.

A sample from one randomly selected animal was chosen to see how the volume estimates of three grass species varied with number of transects examined (Fig. 2). For the key species, *Saccharum spontaneum*, approximately 13 transects were needed to estimate a volume within 90% of the mean of 25 transects. For moderately preferred (*Imperata cylindrica*) and less preferred (*Vetiveria zizanoides*) species more than 20 transects were required for a similar level of precision (Fig. 2).

Data from the same animal were used to compare volume composition based on frequency distribution of fragment interception and estimates based on actual measurements of individual fragments (Table 2). The results showed very close agreement for all species, and the correlation was highly significant ($r^2 = 0.99$, $P < 0.001$).

Variation between individual animals

A pooled sample of fifteen randomly collected samples obtained more than 90% of the total number of species collected from 20 unknown animals. In the case of known animals a pooled sample of ten produced the same range of species in the diet.

So far, the number of fecal samples required in order to establish the food habits of a megaherbivore like rhino has not been documented. Anthony and Smith (1974) suggested that a minimum of 15 fecal samples are required for studying deer diets within a particular

period. But they did not mention whether the same number of fecal samples from the same or different animals provided similar results.

The discrepancy in the results between known and unknown animals in the present study may have occurred because some fecal samples collected from unknown animals could have been duplicated by the same animal. Rhinos use common latrines and defecate on the same spot several times in a twenty-four hour cycle (Laurie, 1978). In the present study all fecal samples of unknown animals were collected in early morning from latrines located within an area of approximately 2 km^2, and at each latrine 2 or more dung piles that had been defecated during the preceding night were collected. This might have led to some duplication of fecal samples from the same animal. Thus, it is suggested that if fecal samples are collected from unknown animals a pooled sample from at least twenty different latrines is required in order to represent the total range of food plants. Two grasses and one browse species were selected to see how the variation in volume estimates varied with number of individuals sampled. The results showed that key food species were less sensitive to the sample size than were moderately and less preferred species (Fig. 3). For key species (*Saccharum spontaneum*, *Narenga porphyrocoma* and *Trewia nudiflora*) samples from 4-5 different animals gave results within 10% of the volume estimate of ten animals, while at least 8 and 9 samples were required for the same level of precision for moderately preferred (*Phragmites karka*, *Saccharum bengalensis* and *Callicarpa macrophylla*) and less (*Typha elephantina*, *Chrysopogon aciculatus* and *Mallotus phillippinensis*) preferred species, respectively.

The diet volume composition from pooled samples of 5 and 10 animals, and the mean of five different animals were compared with the mean diet composition of ten different animals (Table 1). The two pooled samples both fell within 95% confidence limits of the

mean of 10 different animals. The range of variation in five random combination of five different animals was also low for key species and higher for medium and less common species (Table 1). Among key species the range was 9.8%-19.9%, 19.3%-36.4%, and 24.9%-37.1% for *Trewia nudiflora*, *Saccharum spontaneum* and *Narenga porphyrocoma*, respectively.

Volume estimates were also compared to see how accuracy is influenced by pooling samples. Highest variation was recorded among the less preferred species (Fig. 4). Precision for moderately and less preferred species increased slightly by pooling the samples from 5 to 10, however, none of these increments were statistically significant. Besides, the mean of 5 different animals also did not provide better estimates than the pooled sample of 5 random animals.

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Table 1. Comparison of the relative proportion of the diet composition between ten individual samples and pooled samples.

	Mean composition of 10 different animals		Range of variation (%) of 5 random combinations of 5 diff. animals	Mean of 5 different animals		Pooled of 10 animals	Pooled of 5 animals
	%	CV		%	CV	%	%
Grass species							
Saccharum spontaneum	22.7	24.6	19.3 - 36.4	26.5	19.3	21.2	24.5
Narenga porphyrocoma	20.0	30.0	24.9 - 37.1	19.6	30.1	23.2	19.5
Saccharum bengalensis	4.5	106.7	91.7 - 113.9	1.7	223.6	6.3	6.6
Phragmites karka	3.6	116.7	85.1 - 97.5	0.8	137.5	3.1	4.3
Imperata cylindrica	3.5	102.9	98.5 - 113.1	3.9	113.1	3.4	4.8
Themeda sp.	3.3	115.2	101.9 - 224.0	1.0	224.0	3.8	2.2
Saccharum arundinaceum	2.3	52.2	40.8 - 71.7	2.3	71.7	1.2	3.2
Cyanodon dactylon	2.2	159.1	96.0 - 146.5	3.1	146.5	3.5	2.1
Vetiveria zizanoides	1.5	100.0	64.4 - 107.2	1.5	107.2	0.7	0.8
Seteria sp.	1.1	118.2	94.0 - 224.2	1.2	110.5	0.9	0.8
Desmostachia bipinnata	1.0	160.0	112.1 - 164.2	1.3	134.4	1.3	0.0
Chrysopogon aciculatus	0.9	188.9	146.2 - 222.2	1.6	146.2	0.3	0.0
Typha elephantina	0.7	171.4	13.3 - 223.5	1.3	113.3	0.6	1.0
Cymbopogon sp.	0.4	220.0	223.1 - 225.0	0.3	225.0	1.0	0.0
Panicum sp.	0.5	220.0	223.8 - 224.2	0.4	223.8	1.1	0.0
Browse species							
Trewia nudiflora	13.4	17.9	9.8 - 19.9	14.4	19.9	11.7	15.3
Callicarpa macrophylla	4.2	52.4	39.9 - 81.4	3.3	63.7	3.4	3.4
Ehretia laevis	1.8	150.0	119.9 - 158.8	1.7	158.8	2.0	0.0
Colebrookia oppositifolia	1.6	162.5	100.0 - 146.2	3.1	100.0	0.3	0.9
Murraya paniculata	0.7	142.9	114.5 - 213.8	0.4	176.2	0.2	0.5
Bahunia sp.	0.4	225.0	158.8 - 224.1	0.8	158.8	0.2	0.0
Mallotus pillippinensis	0.4	225.0	223.5 - 224.1	0.5	224.1	0.6	0.0
Others							
Cyperus sp.	1.6	112.5	88.1 - 162.5	2.3	88.0	1.1	1.2
Circium wallachii	0.8	187.5	152.3 - 225.0	0.4	225.0	1.6	0.3
Urena lobata	0.7	185.7	146.5 - 222.5	0.4	222.5	0.1	0.9
Pteris sp.	0.6	100.0	73.8 - 104.4	0.5	95.8	1.0	1.3
Truimfetta sp.	0.3	233.3	171.7 - 224.0	0.1	225.0	0.2	0.0
Artemisia vulgaris	0.2	400.0	0.0 - 222.9	0.5	222.9	0.1	0.0
Unidentified	5.6	21.6	18.1 - 27.5	5.4	25.9	5.9	6.4

...l samples and pooled

...led 0 ...nals	Pooled of 5 animals
	%
	24.5
	19.5
	6.6
	4.3
	4.8
	2.2
	3.2
	2.1
	0.8
	0.8
	0.0
	0.0
	1.0
	0.0
	0.0
	15.3
	3.4
	0.0
	0.9
	0.5
	0.0
	0.0
	1.2
	0.3
	0.9
	1.3
	0.0
	0.0
	6.4

Table 2. Diet composition based on actual measurements of fragments and on the freque... distribution of number of intercepted fragments from microfecal analysis of one rando... selected animal.

Plant species	Volumetric Measurements Volume (%)	SE	Frequency distribution No. of fragments	f(%)	S
S. spontaneum	22.3	1.4	177	21.6	2.
N. porphyrocoma	20.2	1.5	165	20.1	1.
T. nudiflora	15.7	1.8	121	14.7	1.
S. bengalensis	9.6	1.1	87	10.6	1.
I. cylindrica	6.1	1.1	57	6.2	0.
P. karka	4.8	0.9	36	4.4	2.
Themeda sp.	4.4	1.7	27	3.2	2.
C. macrophylla	3.7	0.7	30	3.7	1.
S. aurandineceum	2.5	1.3	23	2.8	1.
V. zizanoides	2.1	1.5	17	2.1	2.
Cyperus sp.	1.0	0.7	23	2.8	1.
Pteris sp.	1.1	0.8	14	1.7	1.
Unidentified	6.5	0.6	50	6.1	1.
Sum	100		827	100	

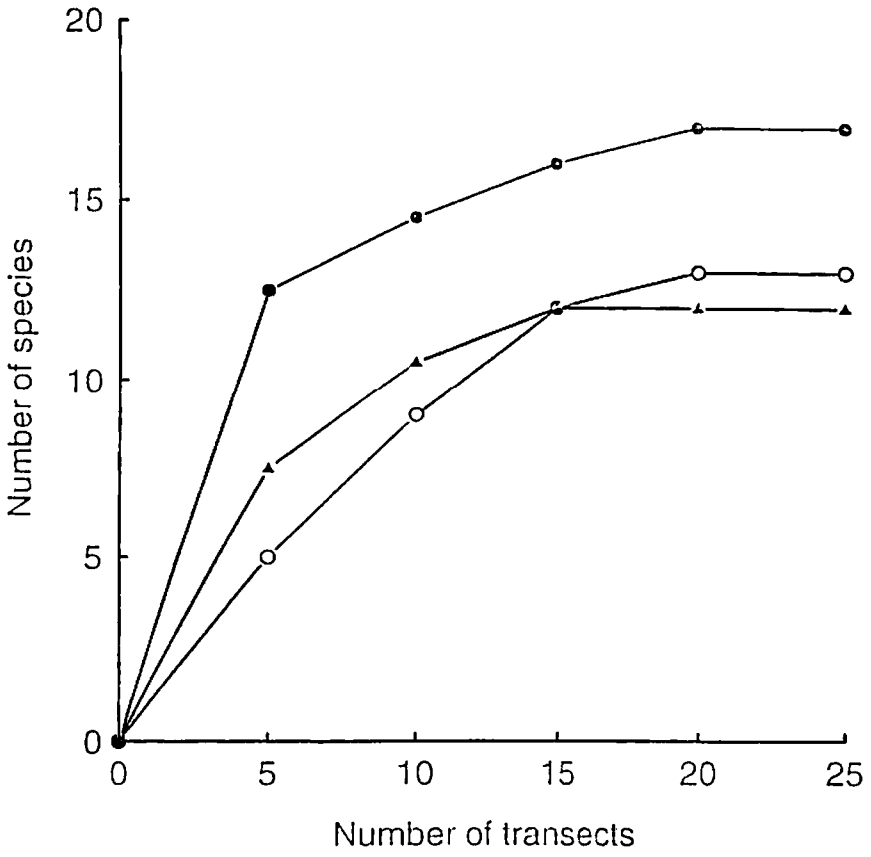

Fig. 1. Relationship between number of species recorded and number of transects examined based on fecal samples from three different animals.

Fig. 1. Relationship between number of species recorded and number of transects examined based on feed samples from three different animals.

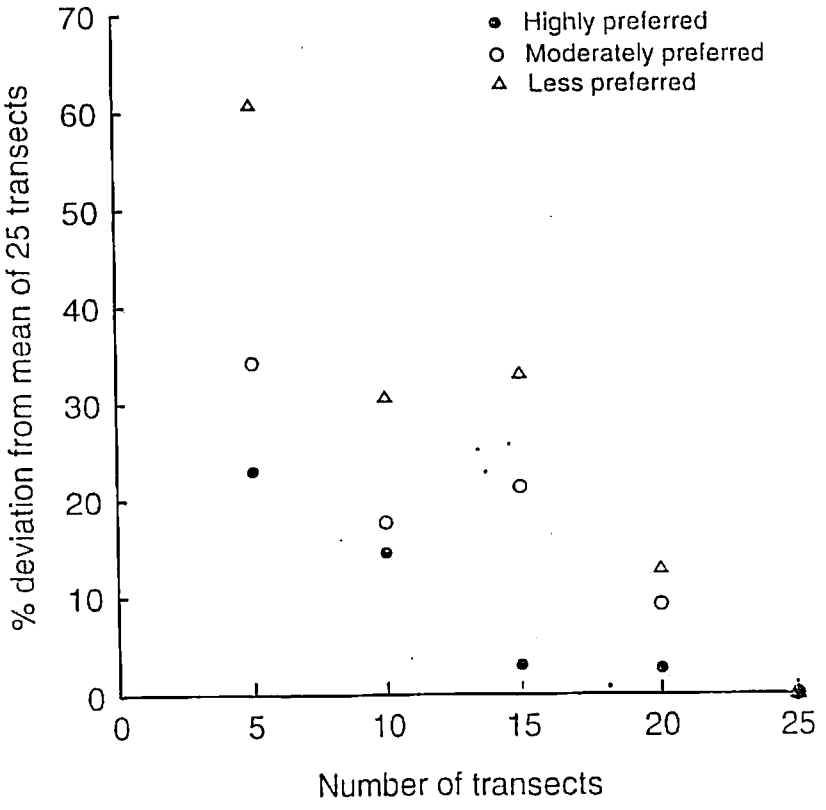

Fig. 2. Relationship between volume estimates of three grass species and number of transects examined.

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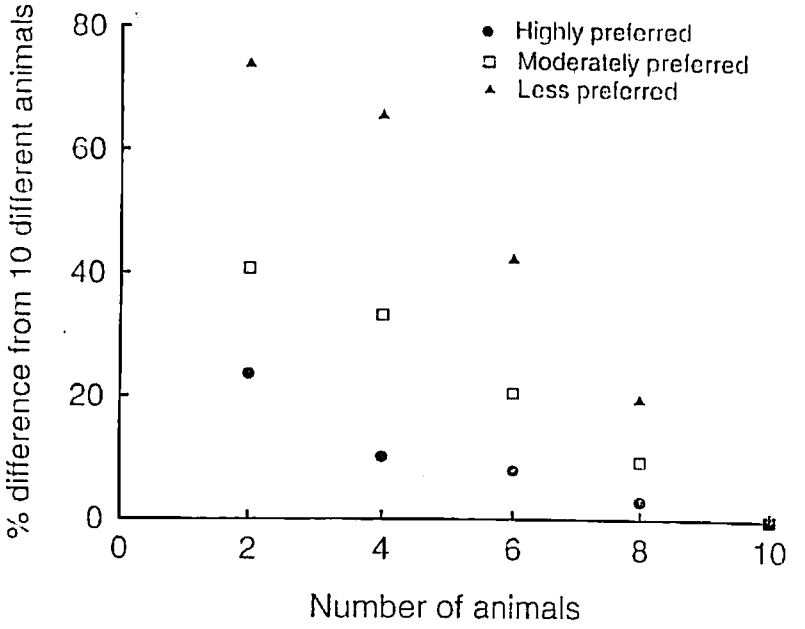

Fig. 3. Relationship between number of different animals sampled and volume estimates of different categories of food plants in the diet.

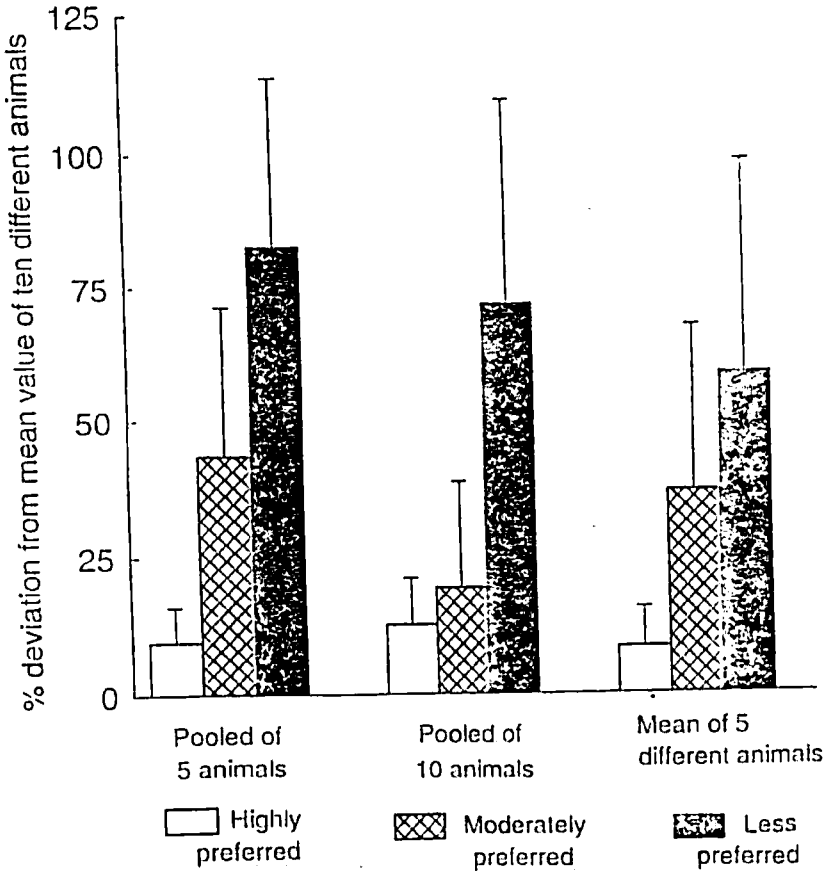

Fig. 4. Relationship between percent deviation in volume estimates of pooled samples from mean value of ten known different animals. Vertical