A REGIONAL COOPERATIVE DAPA RESEARCH AND DEVELOPMENT PROGRAM

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Abstract: Levels of amino acid residue of rumen fermentation (DAPA-diaminopimelic acid) recovered in feces of elk and other large ruminants may be a useful criterion for rapid and inexpensive monitoring of the nutritional well-being of these animals. This residue (DAPA) passes unabsorbed through the digestive process with no measurable loss, and passes out in the feces. Like the seasonal changes in diet digestible energy which it reflects, fecal DAPA follows an annual cycle: low when diet quality is low, such as in winter, and high when diet quality is high, such as early in the forage growing season. Due to the method's simplicity and relative low cost its greatest application may be in extensive monitoring programs for identifying herds with apparent nutritional problems. A cooperative research program has been initiated to coordinate investigations of DAPA's potential in this regard. Objectives include: (1) documentation of relationships between diet energy digestibility and fecal DAPA levels for the major North American wild ruminant species, and (2) development of regional fecal DAPA profiles for these species, using samples submitted by state and provincial cooperators. Recent unpublished results from cooperative state efforts and controlled feeding studies of penned animals are presented and discussed.

The DAPA method (Nelson et al. 1982, Davitt and Nelson 1984) may be useful in extensively evaluating the nutritional well-being of wild ruminant populations. The method monitors a unique fecal amino acid, 2,6 diaminopimelic acid (DAPA), which is correlated with dietary protein and digestible energy (DE) in domestic sheep (Nelson et al. 1983), and exhibits seasonal patterns (profiles) in mule deer and elk like DE (Nelson et al. 1982, Davitt and Nelson unpublished). DAPA is found in the cell walls of rumen bacteria and eventually in the ruminant's feces, because it is neither digested nor absorbed by the ruminant.

The North American cooperative DAPA research and development project was initiated to coordinate investigations of DAPA as a criterion for monitoring the nutritional well-being of big game herds. The project was approved for funding by the North American Association of Fish and Wildlife Agencies in September, 1983, and was cooperatively funded by the
U. S. Fish and Wildlife Service and Washington Agricultural Research Centre in March, 1984. Objectives of this project have been to:

1. initiate and coordinate a cooperative research effort to
   
a. document relationships between diet digestible energy and fecal DAPA levels for at least the major North American wild ruminant species through controlled feeding studies.

   b. develop regional fecal DAPA profiles for these species, using field collected samples submitted by cooperating resource agencies.

Quantity and quality of food is generally considered the principal constraint of big game herd productivity (Connolly 1981, Nelson and Leege 1982) and subsequent harvestability, and much research and management effort has been focused on this subject area. In spite of this, all too frequently we remain ignorant of the animal's nutritional needs. Standards for the nutritional requirements of wild ungulates are as yet only crude approximations drawn heavily from the livestock literature.

Many, including these writers, have accepted available nutritional standards as adequate and have evaluated how well the habitat provides for the protein and energy needs of the animal (e.g., Schommer 1978, Gibbs 1978, Davitt 1979, Hobbs 1979, Hobbs et al. 1981, Baker and Hobbs 1982). Some have proceeded even further by estimating carrying capacity on a nutritional basis (Wallmo et al. 1977, Hobbs et al. 1982). However, this requires accurate determination of food habits and digestible protein and energy, either on a forage species-by-time or diet mixture-by-time basis. All methods for quantifying food habits continue to have serious technical and logistic problems (Medin 1970, Rice 1970, Ward 1970, Wallmo et al. 1973, Bartmann and Carpenter 1982, Nelson and Leege 1982). Even with accurate food habits data, it is necessary to express them in terms of digestible nutrients. This is an expensive procedure, even when using the least expensive methods; vis., food habits determined by fecal analysis with forage samples in diet mixture subject to in vitro digestion of crude protein and gross energy. At best, current methods for evaluating big game food habits on a nutritional basis are expensive and only marginally reliable.

Many biologists favor animal indicators for monitoring the well-being of big game populations because they are more direct than the usual diet quality approach and simpler, with less opportunity for technical errors to bias results and their interpretation. Some of the more commonly used indicators include ovarian analysis and fetal counts (e.g., Robinette et al. 1977), bone marrow color (Cheatum 1949, Hunt 1979, Peterson et al. 1982) or compression (Greer 1968), kidney fat index (Riney 1955), blood urea (Warren et al. 1982), and combinations of indicators (Ransom 1965). Although useful, these indicators evaluate productivity and animal condition after the fact and require the death of the animal. Direct methods for evaluating current nutritional well-being which have been used involve nutrient analysis of the rumen for crude protein (McBee 1964 cited by Moen 1973:312) or volatile fatty acid (VFA) production (Mansfield et
These methods also require the death of the animal, unless a trocar is used on darted and drugged animals.

A unique fecal amino acid, that of undigestible portions of bacterial cell walls, shows strong promise of being closely correlated with diet quality (Nelson et al. 1982). The compound, 2,6 diaminopimelic acid (DAPA), is found in most anaerobic bacteria and blue-green algae but not in higher plants and animals. The ratio of DAPA to total bacterial nitrogen in a mixed rumen bacteria population has been shown to be rather consistent. Because of this, DAPA has been used as a marker for determining nitrogen flow to the lower tract of ruminants (Hogan and Weston 1970, Hutton et al. 1971, Lindsay and Hogan 1972). Once diet nitrogen is combined into DAPA, it passes through the animal undigested and unabsorbed (Hutton et al. 1971). Since at least 80% of a grazing ruminant's digestible energy is derived from bacteria-produced VFA and digested bacterial constituents (Weller 1969) and, since DAPA comprises an indigestible proportion of the bacterial mass, it follows that fecal DAPA could be strongly correlated with diet digestible energy.

DAPA was first isolated from Corynebacterium diphtheriae by Work (1951) and has subsequently been found in cell walls of most rumen bacterial species tested, as well as blue-green algae (Work and Dewey 1953, Syngue 1953, Hoare and Work 1957, Purser and Buechler 1966). It has not been found in other algae, fungi, plant viruses, protozoans, and higher plants (Larsen and Norris 1976), except as bacterial contamination (Rhlund 1960, Czerkawski 1974). Theurer (1982) found DAPA levels in protozoa and higher plant material which were too high to be explained by bacterial contamination but might have been due to elution of unknown amino acids with DAPA. All three possible stereoisomers of DAPA have been isolated from bacteria, but the meso- and LL-forms are most prevalent (Hoare and Work 1957).

Following the early efforts of Weller et al. (1958), DAPA has been most commonly used as a criterion for separating bacterial nitrogen from undigested forage residue nitrogen in the upper GI tract (El-Shazly and Hungate 1966, Freitag et al. 1970, Mason 1969, Dufva et al. 1982, Verma and Srivastava 1980). For DAPA to be used to predict rumen bacterial content, concentrations of DAPA in bacteria must be constant or its variation be understood and predictable. Some workers have assumed a constant DAPA-nitrogen in the bacterial nitrogen fraction and Hutton et al. (1971) showed that the DAPA/nitrogen ratio was constant for a constant feeding regime.

Since DAPA concentration in the upper GI tract could be used to estimate average net bacterial biomass growth to about 5% accuracy (El-Shazly and Hungate 1966), subsequent workers have used DAPA concentrations in undigested forage material throughout the GI-tract (including feces) to evaluate diet quality. Freitag et al. (1970) and Virtanen (1966), working with cattle, found significant increases in DAPA concentrations when diet quality was enhanced with urea supplements. Mason (1969) found significant variation in fecal DAPA concentration among sheep fed a variety of forages and supplements. DAPA concentration was not correlated with diet crude protein, however.
Czerkawski (1974) found significant changes in rumen bacterial numbers and types with varying levels of linseed oil supplemented to sheep diets, as well as corresponding changes in rumen DAPA and total nitrogen content. DAPA increased with increased oil supplement to mid-levels, then decreased. He attributed DAPA increases to bacterial mass increases. Above mid-level oil supplementation, Selenomonads and other large bacterial species increased disproportionately, with resulting decreases in DAPA concentration.

Nelson et al. (1982) were first to examine relationships between fecal DAPA and digestible energy (DE) in the diet. Using sheep fed a variety of high quality feeds with concentrates, they showed that fecal DAPA was directly related to diet DE. Additionally, using a DE estimator derived from this sheep study, they found that fecal DAPA might be useful in estimating diet DE for elk and other wild ruminants and suggested that DAPA profiles (fecal DAPA values over time) could be an important tool in monitoring the nutritional well being of free-ranging wild ungulates. They called for cooperative research among the state and federal agencies to investigate this possibility.

METHODS

Wildlife nutritionists from the United States and Canada will collaborate in documenting relationships between diet digestible energy and fecal DAPA. Controlled feeding studies of subject species will be carried out in digestion metabolism stalls or other appropriately designed structures in which quantity of feed and water consumed and total feces and urine voided can be monitored. Rations fed should contain varying amounts of natural, high fiber forage of varying quality to simulate as closely as possible diets of free-ranging animals. Consumption levels and forage quality should be closely controlled, but varied. Each treatment should be replicated at least twice.

In a controlled feeding study with domestic sheep, Nelson et al. (1983, unpublished) varied both consumption rate and forage quality. They found that, not only was forage quality reflected in the fecal DAPA levels, but that DAPA levels were also influenced by consumption rate. Highest correlation \(r = .980\) was shown between fecal DAPA level and total daily digestible energy intake (Figure 1).

Further method validation research will be initiated with cattle feeding experiments during the summer of 1984 and with mule deer in the spring of 1985. At present, facilities for the mule deer controlled feeding trial are being constructed. Fawns are being halter-trained to accustom them to confinement in pens and metabolic crates.

Chopped, native forages will be fed ad libitum in mixtures which will simulate mule deer winter diets in central Washington. Diet digestible energy, protein, and fiber fractions will be compared to DAPA and other fecal parameters for each feeding trial.

State game management agencies scattered throughout the ranges of the subject animals will be solicited for their cooperation in this study.
Figure 1. Relationship between digestible energy intake vs. fecal DAPA for sheep. Data from Nelson et al. (1983).
They will be asked to participate for at least three years, the anticipated minimum time needed to sample for variations in amplitude and wavelength expected among annual DAPA profiles for a herd.

Fecal DAPA profiles will be monitored on at least a monthly basis for herds of subject animal species with contrasting nutritional planes. Ideally, paired herds could be selected from within a limited geographic area to minimize variation associated with geographic and climatic differences: one herd which, in the opinion of local specialists, has near optimal nutritional conditions, the other in poor to fair nutritional circumstances. Of course, paired herds will not be required when more than two herds with contrasting nutritional background are selected from the same general area. Herd selection will be entirely up to the discretion of the cooperating agencies.

RESULTS AND DISCUSSION

Fecal DAPA monitoring has begun for 74 wild ruminant herds, including 12 white-tailed deer herds (on for Columbian whitetail), 18 mule deer herds, 10 black-tailed deer herds, 10 Rocky Mountain elk herds, 8 Roosevelt elk herds, and 6 California bighorn sheep. Others include Tule elk, moose, Stone sheep, pronghorn, barrenground caribou, woodland caribou, Sanbar deer (Guam), and water buffalo (Guam). This effort is being carried out by 35 cooperators in 16 states and Canadian provinces.

To date, a limited number of herds have been monitored for a year or more for fecal DAPA, most of them have been elk herds. Results, however tentative, reinforce our earlier (Nelson et al. 1982) suppositions that herd fecal DAPA levels (1) are cyclic, at least for northern herds, being low in winter when diet quality is expected to be low, and high in late spring and early summer when diet quality is expected to be high; (2) vary in pattern between years, (3) vary among animal species, and (4) vary within species.

Elk. Fecal DAPA patterns have been monitored for 5 elk herds for over a year in Idaho, Washington, and California. The Northwestern herds are Rocky Mountain subspecies, and the California herd is Tule elk. In Washington, the Mt. St. Helens (R. Tabor unpublished) and Yakima (W. Meyers unpublished) herds showed similar DAPA profiles in late 1982 and through 1983 (Figure 2A). DAPA levels remained consistently high from May through August for both herds and were lowest in December. The most obvious difference between habitats of these herds is that the Yakima herd winters at lower elevations where the range is more herbaceous in nature and is free of snow most of the winter. The Yakima herd's better winter range and access to winter feeding facilities may be reflected in their higher winter DAPA levels.

Figure 2B compares the 1982-1983 fecal DAPA profile of a Blue Mountain, Washington, elk herd (W. Meyers unpublished) with that of two composited northern Idaho elk herds (T. Leege unpublished). Both herds summer in mixed-conifer habitat, but the Washington herd winters in open sagebrush/bunchgrass to semi-open ponderosa pine mostly below 2000 feet, while the Idaho herd winters in coniferous forest and seral brush fields
Figure 2. Fecal DAFA profiles for selected Rocky Mountain elk herds in Washington and Idaho as well as Tule elk in California. Unpublished data credits: Blue Mountains and Yakima herds, W. Meyers; Mt. St. Helens herd, R. Tabor; North Idaho herds, T. Leege; Tule elk herds, M. Hanson.
above 3000 feet elevation. Spring greenup and, subsequently, improved forage quality frequently begins in March on the Blue Mountain herd's winter range, but is delayed until May for the Idaho elk. This appears to be reflected in the DAPA profiles for the two herds.

Tule elk (M. Hanson unpublished) in Monterey County, California, have shown the most remarkable, yet not unexpected, DAPA profile for elk (Figure 2C). This small subspecies of elk showed higher DAPA levels than the larger subspecies, ranging from a low of about .6 mg/g to highs of nearly 1.4 mg/g in 1982 and 1.7 mg/g in 1983. These DAPA levels are more similar to white-tailed and mule deer than they are to other subspecies of elk. Lowest DAPA levels were observed in September and October, the end of summer drought in that region; highest were observed in winter and spring, their period of active plant growth.

No Roosevelt elk herds have been monitored long enough to characterize their DAPA profiles. Results of analyses conducted on fecal samples from this species suggest that their DAPA levels will be similar to those of the Rocky Mountain elk.

Mule and Black-tailed Deer. Nearly all cooperators working with these two subspecies initiated field work late in 1983 or in 1984. Consequently only two black-tailed deer herds (Kie and Burton 1984) have been sampled for a full year. These deer largely summer in coniferous forest and migrate down to oak-brush and lower elevational coniferous forest in winter. These workers found distinct monthly variation in both fecal nitrogen and DAPA levels (Figure 3) but no significant DAPA differences between the two herds. Highest DAPA levels were observed in June; lowest occurred in late fall and winter. A secondary DAPA peak was observed in December for both herds, which the authors attributed to acorn consumption. Kie and Burton recommended further study to related seasonal range use and food habits to fecal nitrogen and DAPA.

White-tailed deer. The Florida Game and Fresh Water Fish Commission, beginning in October, 1982, has the longest tenure of cooperation with this project of any of the white-tailed deer cooperators, as well as being the only cooperator with at least one year's field study. Five herds were sampled (F. Smith unpublished), although DAPA analysis is incomplete at this time. DAPA profiles for 1982-1983 for four herds are presented in Figure 4. The Edward Ball and St. Regis herds, located in the Florida Panhandle region, showed lower DAPA levels for spring and summer than the Osceola and Ocala herds which were located in the Central Highlands. The St. Regis herd has historically been the more productive of the two Panhandle herds and showed a higher spring and summer DAPA level. The Highlands herds have shown similar productivity through the years, but the Osceola herd shares its range with 1,650 head of cattle.

Although only winter months were sampled on four white-tailed deer areas in western Montana (C. Seeley unpublished), preliminary data from this study have merit for presentation here, because they demonstrate a different use for fecal DAPA monitoring studies. Seeley sampled fecal DAPA on two areas currently being logged, and two unlogged areas, from December, 1983, to March, 1984, as well as determined monthly white-tailed deer food habits on the same areas, using fecal analysis technique. DAPA
Figure 3. DAPA profile for black-tailed deer from Trinity County, California (Kie 1983).
Figure 4. Fecal DAPA profiles for white tailed deer in (B) Northwestern and (A) Central Highland Regions of Florida for the period beginning November, 1982, and ending September, 1983 (F. Smith unpublished.)
content of feces collected on the four areas during the 1983-1984 winter are presented in Figure 5. Deer concentrated in the logged areas, feeding on tops of felled trees. Food habits results showed no significant differences in diet composition between logged and unlogged areas in December and January. Conifer browse, mainly Douglas-fir, comprised 30 to 50 percent of the diets in all areas. Quality of the conifer browse differed, however. Tree-top Douglas-fir browse averaged a full percent higher (6.3 vs. 5.3) in crude protein than that from lower branches. In addition, logged areas afforded earlier spring greenup, and this resulted in greater green herbage consumption in February and March in those areas. Winter DAPA profiles for the four areas reflect the expected diet quality differences.

Desert Bighorn Sheep. G. Miller (unpublished) compared bighorn food habits, forage quality, and various other habitat evaluation parameters for three bighorn populations in Arizona, as well as monitored fecal DAPA. Range area size, forage composition, and water availability were similar for the three areas. The Dome Rocks herd, having the lowest population and occupying what Miller felt was the poorest range area, showed the lowest DAPA profile (Figure 6). Of the two remaining herds, North Plomosa has been generally larger than the New Water Herd, 125-200 vs. 80-150 sheep. The New Water herd consistently showed higher fecal DAPA through the 1982-1983 sampling period than the North Plomosa herd. Herd productivity statistics for 1983-1984 have not yet been made available.

CONCLUSIONS

Fecal DAPA follows an annual cycle, reflecting the seasonal change in diet digestible energy: low when diet quality is low and high when diet quality is higher. Due to the methods simplicity and relative low cost, the greatest application of DAPA analysis may be in extensive monitoring programs for identifying big game herds with apparent nutritional problems.

Efforts will continue to collaborate with other researchers which have initiated DAPA method validation research (feeding trials), and to seek to enlarge the number of big game species being studied. Feeding trials using mule deer will be conducted by these authors to document the relationship between DAPA and diet digestible energy, protein and fiber fraction.

Currently, fecal DAPA monitoring has begun for 74 wild ruminant herds being carried out by 35 cooperators in 16 states and Canadian provinces. New cooperators are anticipated and encouraged to join these cooperative research efforts. Although only a limited number of herds have been monitored for a complete year to date, results reinforce earlier suppositions that herd fecal DAPA levels (1) appear cyclic, (2) may vary in pattern between years (plant phenology, climatic conditions), (3) may vary among animal species, and (4) may vary within species.

Ongoing studies include determining the effects of weathering, storage, and handling on DAPA levels in selected wild ruminant fecal samples; to evaluate pellet group DAPA variability to optimize sampling
Figure 5. Fecal DAPA profiles for wintering white-tailed deer on two areas currently being logged (L) and two unlogged control (C) areas in western Montana (Seeley unpublished).

Figure 6. Fecal DAPA profiles for three desert bighorn sheep herds in Arizona (G. Miller unpublished).
intensity for major big game species; and to test alternative methods for quantifying fecal DAPA (Davitt and Nelson 1984).

LITERATURE CITED


Tabor, R. 1984. Unpublished. DAPA on elk in the Mt. St. Helens area, WA. Univ. of Washington, College of Forest Resources, Seattle, WA.


