UNIVERSITY OF CALIFORNIA
HOPLAND RESEARCH & EXTENSION CENTER

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THREE-YEAR REVIEW REPORT

Title of Research Project: The use of N-alkanes to determine dietary overlap of sheep and goats in California Chaparral

Center Project No.: 47-00

AES or CEFS Project No. and Title: CA-D*-ASC-6740-H

Project Leader: Wolfgang Pittroff, Assistant Professor
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Project Cooperators:
(Name & Title)

Attach a detailed 2-4-page narrative addressing the following items:

1. **Justification and Problem Statement** (Describe the original or modified problem and justification).
2. **Objectives:** (Describe the specific original or modified objectives of the project).
3. **Procedures:** (Include experimental design, data collected, and methods of data analysis).
4. **Summary of Research Results:** (Provide a summary of research conducted on this problem. Include statement of how original or modified objectives were met, and relevance of research results to California)
5. **Extension of Results:** (Explain how research results were shared, including publications, field days, seminars, etc.).
6. **Description of Research Planned for the Next Three-Year Period:** (Include objectives and procedures).
7. **Publications:** (Also include unpublished reports to industry or funding agencies, in press and in presentation papers. List and provide single copies).
8. **Attach a completed Land, Labor and Facilities Form.**

Project Leader Signature:_____ Date:_____

I have reviewed this proposal and approve it as appropriate research for the Project Leader and the Center.

Authorizing Signature:_____ Date:_____

The following authorizing signatures are required:

1. For Faculty and Campus Specialists: Department Chair
2. For Farm Advisors: County Director
3. For other State and U.S. agencies: Admin. Unit Head and Cooperating Department Chair

The use of N-alkanes to determine dietary overlap of sheep and goats in California Chaparral.

Justification and Problem Statement.

There is strong interest in the use of domestic ruminants as tools in vegetation management in areas prone to brush fire hazards and exotic weed species invasions (Sverson and Debano, 1991). Indeed, in Europe there are breeds of ruminants specifically selected for the management of characteristic vegetation associations (Haring, 1975).

The effective use of ruminants in vegetation management requires knowledge about dietary preferences and dietary overlap between ruminant species. It is well known that dietary overlap between ruminant species (including both domestic and wild) varies according to properties of the vegetation (species composition, growth stage, stand morphology) (Murray and Illius, 1996). In order to develop pre-scribed grazing strategies for management of Californian Chaparral brush land, the site-specific determination of diet composition and dietary overlap of sheep and goats is required. Such work is an initial step in future inter-disciplinary work studying the effects of mixed species grazing on vegetation development and hydrology of the California Chaparral.

Detailed information about dietary overlap of domestic herbivore species under California Chaparral conditions is sparse. Longhurst et al. (1979) presented detailed data on dietary overlap between deer and sheep, largely collected on the Hopland Station. This information is very valuable for the presently planned experiments, but it is important to point out that the method used (rumen content analysis of slaughtered animals) cannot account for within-animal temporal variation of diet selection. The extent or significance of this variation is unknown. Sidahmed et al. (1981) published information on nutritional properties of selected chaparral species in goats, and Wilson et al. (1970) evaluated nutritive properties of 2 chaparral species in sheep. The dietary overlap between sheep and goats under California Chaparral grazing conditions is unknown. From similar vegetation types it is known that goats tend to prefer woody vegetation, and are known to consume most California Chaparral species (Peischel, pers. comm.). It is likely that mixed species grazing will be an effective tool for maintenance of a desired vegetation matrix. Sheep require less herding effort, and thus should be more cost-effective in long-term vegetation management than goats. However, the control of species unpalatable for sheep will require combination with goats. The method to be used for the determination of diet selection has been applied successfully in similar ecosystems in Europe and the Middle East (Bento et al., 1999; Rothmann et al., 1999). The significance of this work is exemplified by the need of public utility companies in California to consider controlled ruminant grazing as possibly the most effective tool in vegetation management of watersheds subject to extreme fire hazards. An example is the peninsula watershed of the San Francisco Water Department (Ciardi, pers. comm.).

Objectives

The project will research the following hypotheses:

1. Plant wax components can be used as markers to establish diet composition and dietary overlap of sheep and goats.
2. Dietary overlap of sheep and goats varies according to season.
3. Concentrations of plant allelochemicals and their metabolites in rumen fluid

vary between goats and sheep and correspond to intake level.

4. Intake level per unit of body mass and liquid and solid phase digesta kinetics vary between sheep and goats grazing California Chaparral pastures.
5. A protocol for prescribed grazing using sheep and goats and specific to California Chaparral can be developed based on studies of dietary overlap and diet composition.

Procedure

The project will use a mixed flock of sheep and goats (whethers > 1 yr). The flock will contain 8 sheep and 8 goats. Experimental animals will be managed on a pasture representative of California Chaparral vegetation. If plant composition data of the experimental pasture are not available, cover and frequency data will be determined on fixed transects and random plots using standard vegetation inventory techniques (Cook and Stubbendieck, 1986).

Experiment 1 (Hypotheses 1,2):

Samples of all forage and browse species comprising > 2.5% by volume in the diets of sheep and deer in the study of Longhurst et al. (1979) will be collected four times a year (January, April, July, October) and assayed for n-alkane profile according to the procedures of Mayes et al. (1986), Dove (1992) and personal communication with Dove and Brosh.

Experiment 2 (Hypotheses 1,2):

Recovery of naturally occurring alkanes will be estimated with 4 sheep and 4 goats. Both groups will be individually limit-fed a diet composed of alfalfa hay, branches of *Ceanothus interregimus* (deerbrush ceanothus) and *Adenostoma fasciculatum* (chamise) in strictly measured proportions. Fecal output will be estimated by total fecal collection. Feces will be composited for consecutive 24 hour periods. Samples for determination of alkane concentration will be subsampled from aliquots taken from the 24-hour composites. Feed component, refusal and fecal samples will be analyzed for n-alkane concentration as in Experiment 1.

Experiment 3 (Hypotheses 1,2):

Four sheep and goats in the experimental flock will be selected at random for the determination of diet composition. Intake will be measured according to Dove and Mayes (1991) by administration of a known quantity of a synthetic, even-chain n-alkane. A slow-release device will be used in order to minimize disturbance of grazing animals. Diet composition will be determined by chemical analyses of the n-alkane profile of fecal samples collect ed daily over a 7 day period in each season according to a protocol developed by Mayes et al. (1986) and modified by Brosh (pers. comm.). Measured n-alkane profiles in feces and plant specimens will be used in the determination of diet composition. Calculations will be performed with the least squares optimization procedure proposed by Dove and Moore (1995). An alternative procedure (maximum likelihood estimation according to distributional properties of multiple markers, a mixture distribution problem) will be explored. Dietary overlap will be determined by overlay of goat and sheep diet composition profiles, according to an equation proposed by Schoener (1968).

Experiment 4 (Hypothesis 3):

Rumen liquid samples will be collected from experimental animals 1 and 4 hour(s) after morning grazing bouts by stomach tube and analyzed for concentration of plant secondary compounds and their metabolites. Sample collection will take place on

three alternating days, such that there will be 6 samples per animal. In this project, only a preliminary screening of differences between sheep and goat in rumen concentrations of plant secondary compounds and their metabolites can be accomplished. We will concentrate on the compounds present in the three most prevalent forage species in the diet of the experimental animals. Since this information will be determined in the course of the experiment, no further details can be provided here. This part of the project will be conducted in collaboration with Dr. M. Friedman, Research Chemist, USDA-ARS Western Regional Research Center, Albany, CA. Analytical methods will be based on Harborne (1991), Hagerman and Butler (1991) and Scheline (1991).

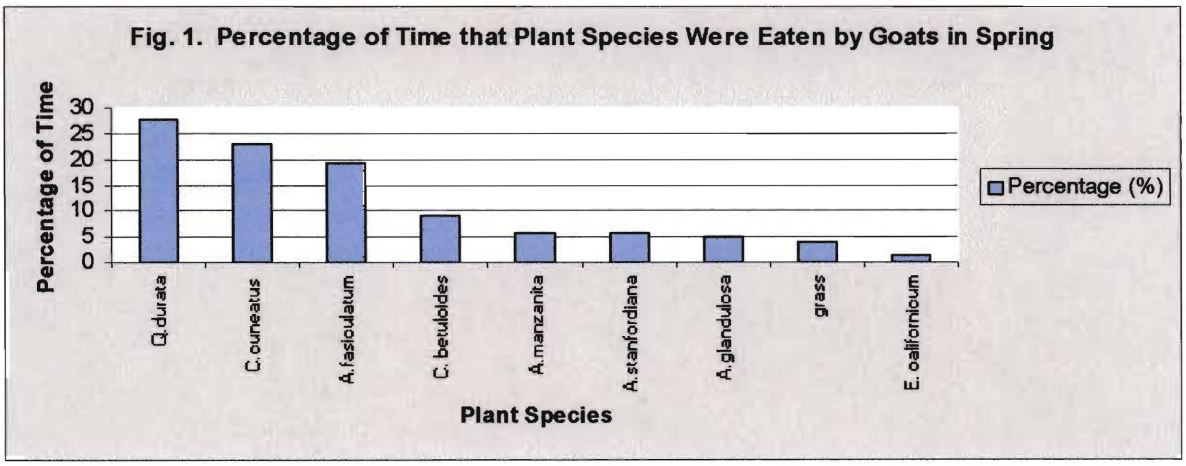
Experiment 5 (Hypothesis 4):

Digestibility and digesta kinetics will be determined using the double marker method of Aharoni et al. (1994). The same experimental animals as in the determination of diet composition will be used, but in different, adjacent time periods. Animals will be dosed with chrome-mordanted NDF and Co-EDTA for marking digesta flow of the solid and liquid phase, respectively, according to procedures described by Aharoni et al. (1994). Fecal grab samples will be collected according to the following schedule: first sample 6 hr pa, second sample 10 hr p.a., then four samples per day in days 1 and 2, three samples per day on day 3 and 4, two samples per day on day 5, 6, and 7. Digesta kinetics will be estimated using a compartmental model proposed by Aharoni et al. (1999). Estimates of fecal output will be combined with intake estimates from Experiment 3 to calculate digestibility values for both livestock species.

Hypothesis 5 will be addressed by summarizing the analysis of seasonal dynamics of dietary overlap.

Summary of Research Results

Experiments 1, 2 and 3 have been completed. All samples are currently in laboratory analysis. The analysis of alkane profiles by GC is time-consuming and expensive. In addition to the experiments above, two seasons of direct grazing observations were conducted to obtain data for validation of the alkane method. Its summary is presented below.



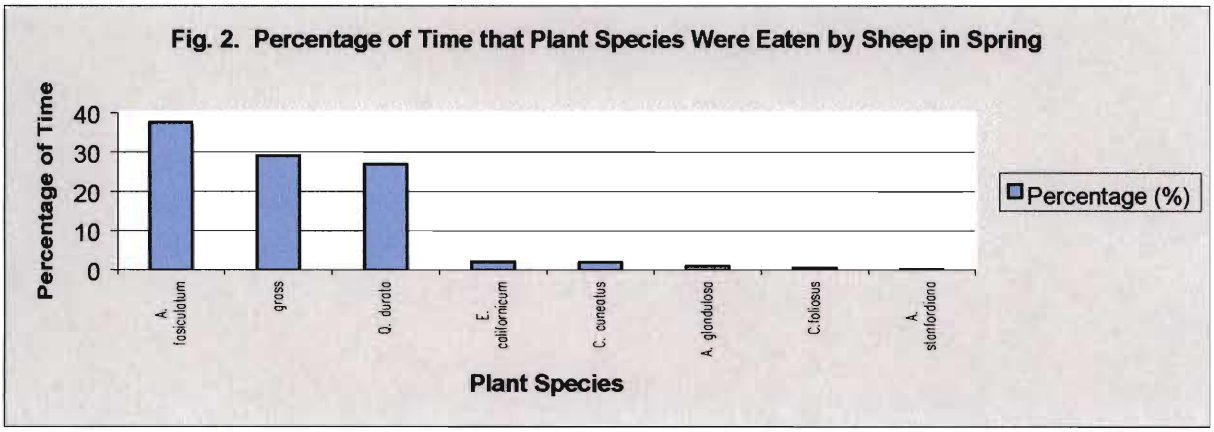


Fig. 2. Percentage of Time that Plant Species Were Eaten by Sheep in Spring
Percentage of Time
40
30
20
10
0
A. fasiculatum
grass
Q. durata
E. californicum
C. cuneatus
A. glandulosa
C.foliosus
A. stanfordiana
Plant Species
Percentage (%)

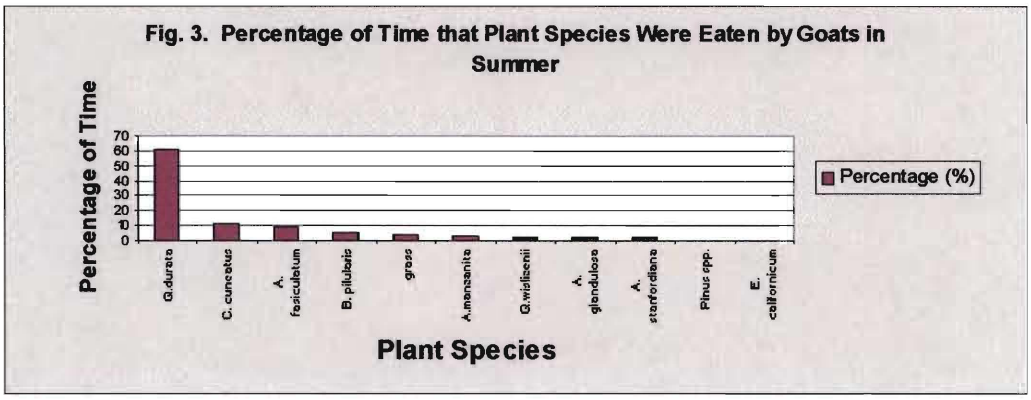


Fig. 3. Percentage of Time that Plant Species Were Eaten by Goats in Summer
Percentage of Time
70
60
50
40
30
20
10
0
Q.durata
C. cuneatus
A. fasiculatum
B. pilularis
grass
A.manzanita
Q.wislizenii
A. glandulosa
A. stanfordiana
Pinus spp.
E. californicum
Plant Species
Percentage (%)

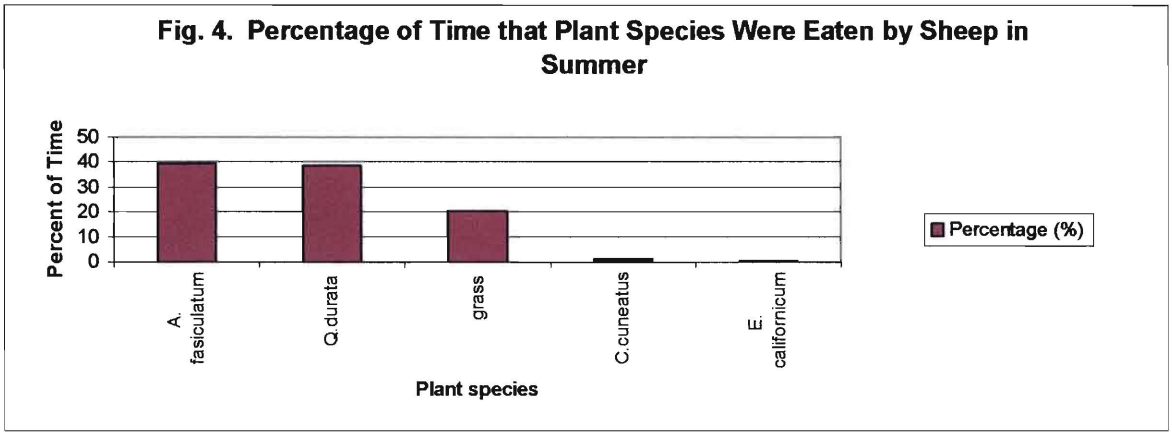


Fig. 4. Percentage of Time that Plant Species Were Eaten by Sheep in Summer
Percent of Time
50
40
30
20
10
0
A. fasiculatum
Q.durata
grass
C.cuneatus
E. californicum
Plant species
Percentage (%)

Fig. 5. Percentage of Plant Species Eaten by Goats in Spring and Summer

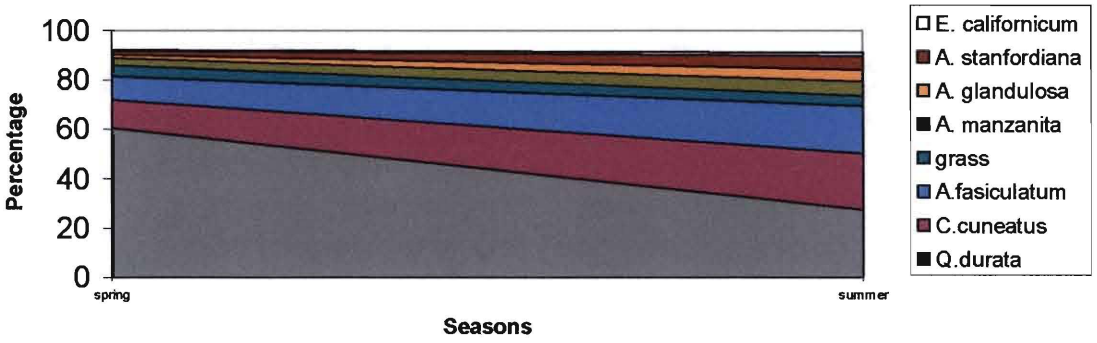


Fig. 6. Percentage of Plant Species Eaten by Sheep in Spring and Summer

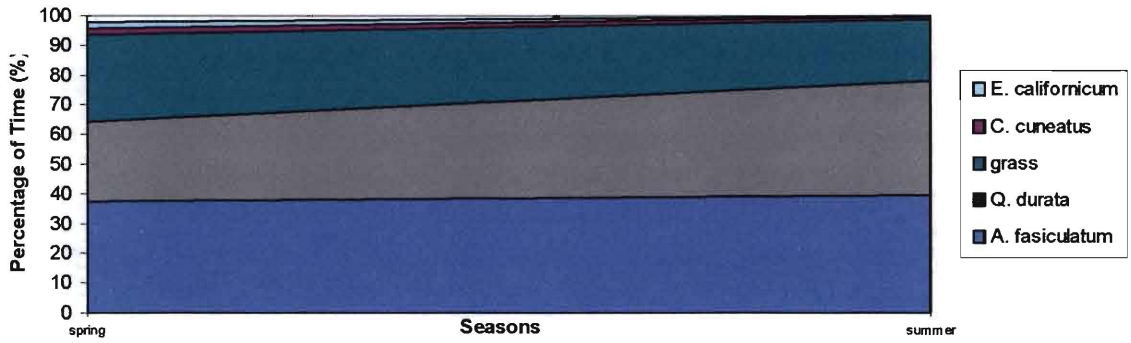
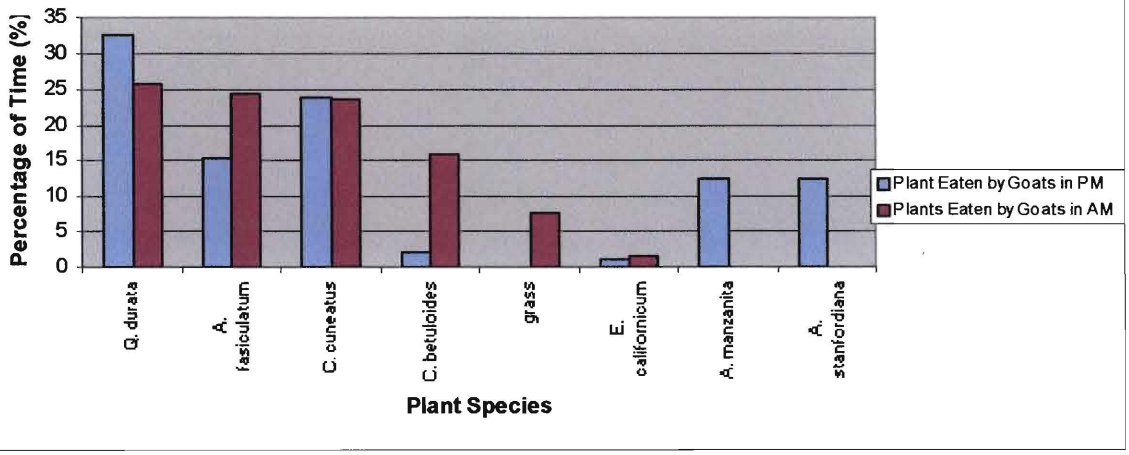


Fig. 7. Percentage of Plants Eaten by Goats at 6:00 and 18:00 in Spring



Data on diet selection (percent time spent eating species *a* of total time of each observation period) were analyzed by analysis of variance with a mixed model maximum likelihood approach (SAS, 1999) Response variables were transformed using a reverse arcsine transformation. Independent variables in the model included season, observation period, animal (modeled as random effect), plant species, observation day (random effect), and residual error. Because the interaction effect of plant and animal species was significant, the effect of plant species on each level of animal species was tested using a "sliced" effects multiple comparison procedure.

In both spring and summer, goats consumed more browse and browse species than sheep (Table 1). Goats consumed fewer brush species in spring (8 species) than summer (10 species). They spent the same amount of time eating grass in both spring and summer. *Quercus durata, Ceanothus cuneatus,* and *Adenostoma fasciculatum* were the major plants consumed by goats in spring. *Quercus durata* was mainly consumed in summer although there was a greater number of species browsed. Plant species that were not consumed by goats in spring but were consumed in summer were *Baccharis pilularis* and *Pinus* spp. (Fig. 1, Fig. 3, Fig. 5).

Some feeding behavior observed in the goats and not in the sheep was the stripping of bark from *Arctostaphylus* spp. in summer. The goats also spit out the fruit of the *Arctostaphylus* spp. on numerous occasions. A bipedal stance was assumed by goats to reach branches of *Pinus* spp. and *Cercocarpus betuloides.*

In contrast, sheep spent a large proportion of time eating grass in both spring (29.1%) and summer (20.5%). They consumed less grass in the summer probably due to decreased grass availability and nutrition. Sheep were observed in the summer to frequently visit areas where there was grass cover during the spring, but none did so during the summer. They did not consume *Arctostaphylus* spp. during the summer. In both spring and summer, they spent the majority of their feeding time at *A. fasciculatum*, grass, and *Q. durata*. More species of plants were consumed in spring (8 species) than summer (5 species). (Fig.2, Fig. 4, Fig. 6) This may have been due to the inability of sheep to select a variety of plant species to meet their dietary requirements, as the nutrition of the plants decreased during the summer. In the spring, plants that goats consumed but sheep did not were *C. betuloides* and *Arctostaphylus glandulosa*. Goats did not eat *Ceanothus foliosus* during the spring, but sheep did. (Fig. 1, Fig. 2) During the summer, sheep did not eat *B. pilularis, Arctostaphylus* spp., *Quercus wislizenii*, and *Pinus* spp. (Fig. 4)

Using the "sliced" effects procedure, there were significant differences between sheep and goats in consumption of *A. fasciculatum* ($p=0.0038$), *Q. durata* ($p=0.0497$), and grass ($p=0.0692$). The difference in consumption of *E. californ*icum was borderline significant ($p=0.0692$). Although the data (Figures 1-4) clearly showed differences in proportion of plant species in the diets of goats and sheep, animal species and many plant species were not significant and suggest that the number of sampling trials was not adequate.

Table 1

Total feeding time of goats and sheep on different categories of forage expressed as percentage of total feeding time.

	Spring		Summer	
	Goats	Sheep	Goats	Sheep
Browse	96.1	70.9	95.5	79.5
Grass	3.9	29.1	4.5	20.5

Extension of Results.

There is an immediate benefit of the proposed study regarding the possible application of mixed species grazing in areas subject to extreme fire hazards. It can be expected that public utility companies and public and private land managers will become more strongly interested in the use of ruminant grazing as a vegetation management tool once more specific data on diet composition and dietary overlap are known.

Our study is providing the basis for future, more basic research. Among planned grant proposals are: 1) Interdisciplinary research on livestock grazing effects on plant community dynamics and hydrology of California Chaparral; 2) Comparative analysis of basic physiological responses of small ruminants to plant allelo-chemicals and possible mitigation mechanisms; 3) Estimation of cost of vegetation management by application of real time methods for the estimation of metabolizable energy intake.

Description of Research Planned for the next Three Year Period.

Experiment 3 must be continued for at least another full year (three seasons) of observations. Experiments 4 and 5 will be initiated once appropriate animal facilities become available in Hopland. As a major remodeling is planned, this will hopefully be possible in 2003. Direct grazing observation work is also planned to continue, as additional qualified students become available.

Publications

Pittroff, W. and L. Brennecke (2002): Hierarchical analysis of dietary preferences of sheep and goats during two seasons in California Chaparral. Submitted to: VII International Rangeland Congress, Durban, SA

References:

Aharoni, Y., A. Brosh, and Z. Holzer. 1994. Double-marker digestive tract model. Journal of Theoretical Biology. 166:407-417

Aharoni, Y., A. Brosh, and Z. Holzer. 1999. Comparison of models estimating digesta kinetics and fecal output in cattle from fecal concentrations of single-doesed markers of particles and solutes. Journal of Animal Science 77:2291-2304

Bento, M.H.L., S. de Rouville, J.L. Gaubert, and G. Bechet. 1999. The use of n-alkanes as markers to estimate intake in goats grazing Mediterranean rangelands in southern France. In: Nutritional Ecology of Herbivores. Posters and Plenary Discussions and Papers presented at Satellite Symposia and Seminar held in conjunction with the Fifth International Symposium on the Nutrition of Herbivores. Editors: Guy Stone, T.D.A. Forbes, J.W. Stuth, and F.M. Byers. San Antonio TX, April 10-16, 1999. (CD-ROM).

Cook, C.W. and J. Stubbendieck. Range Research: Basic Problems and Techniques. Society for Range Management, Denver. 317 p.

Dove, H. 1992. Using the n-alkanes of plant cuticular wax to estimate the species composition of herbage mixtures. Australian Journal of Agricultural Research 43:1711-1724

Dove, H. and A.D. Moore. 1995. Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. Australian Journal of Agricultural Research 46:1535-1544

Dove, H. and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A Review. Australian Journal of Agricultural Research 42:913-952

Dove, H. and R.W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. Journal of Nutrition 126:13-26

Duncan, A.J., P. Frtus, and S.A. Young. 2000. The effect of rumen adaptation to oxalic acid on selection of oxalic-acid-rich plants by goats. British Journal of Nutrition 83:59-65

Hagerman, A.E. and L.G. Butler. 1991. Tannins and Lignins. In: Rosenthal, G.A. and M.R. Berenbaum. (Eds). Herbivores. Their interactions with secondary plant metabolites. Volume 1. The chemical participants. Academic Press, San Diego. p. 355-388

Harborne, J.B. 1993. Introduction to ecological biochemistry. Academic Press. 318 p.

Haring, F. 1975. Schafzucht. Verlag Eugen Ulmer, Stuttgart. 368 p.

Longhurst, W.M., G.E. Connolly, B.M. Browning, and E. O. Garton. 1979. Food interrelationships of deer and sheep in parts of Mendocino and Lake Counties, California. Hilgardia 47:191-247

Mayes, R.W., C.S. Lamb, P.M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. Journal of Agricultural Science, Cambridge. 107:161-170

Murray, M.G. and A.W. Illius. 1996. Multispecies grazing in the Serengeti. In: Hodgson, J. and A.W. Illius. The ecology and management of grazing systems. CAB International, Wallingford. p.247-272

Sampson, A.W. and B.S. Jespersen. 1963. California Range Brushlands and Browse Plants. Division of Agricultural Sciences, University of California, Berkeley. 161 p.

Scheline, R.R. 1991. Handbook of mammalian metabolism of plant compounds. CRC Press. 514 p.

Schoener, T.W. 1968. The anolis lizards of Bimini: Resource partitioning in a complex fauna. Ecology 49:704-726

Sehested, J., K.K. Breinhild, K. Soegaard, L. Vognsen, H.H. Hansen, J.A. Fernandez, V. Danielsen and V.F. Kristensen. 1999. Use of n alkanes to estimate grass inatek and digestibility in sows. In: Nutritional Ecology of Herbivores. Posters and Plenary Discussions and Papers presented at Satellite Symposia and Seminar held in conjunction with the Fifth International Symposium on the Nutrition of Herbivores. Editors: Guy Stone, T.D.A. Forbes, J.W. Stuth, and F.M. Byers. San Antonio TX, April 10-16, 1999. (CD-ROM).

Sidahmed, A.E., J.G. Morris, L.J. Koong, and S. R. Radosevich. 1981. Contribution of mixtures of three chaparral shrubs to the protein and energy requirements of Spanish goats. Journal of Animal Science 53:1391-1400

Sverson, K.E. and L.F. Debano. Influence of Spanish goats on vegetation and soils in Arizona chaparral. Journal of Range Management 44: 111-117

Wilson, A.D., W.C. Weir, and D.T. Torrell. 1970. Evaluation of chamise (*Adenostoma fasciculatum*) and interior live oak (*Quercus wislizenii*) as feed for sheep. Journal of Animal Science 32:1042-1045