Separation of Ether-Soluble Neutral Extract of a Commercial High-Grain Feed Stimulating Hay Intake in Cattle[a]

M. Shahjalal[1], H. Dohi*, T. Kosako and Y. Hayashi
Department of Animal Production, Chugoku National Agricultural Experiment Station, 60 Yoshinaga, Kawai, Oda, Shimane 694-0013, Japan

ABSTRACT : Appetite stimulant ether-soluble neutral extract of a high-grain compound feed was separated into four fractions with an open column of silica gel chromatograph using four carrier solutions containing n-pentane and diethyl ether as 100:0 (Fraction A), 90:10 (Fraction B), 75:25 (Fraction C) and 0:100 (Fraction D). The stimulative effects of the fractions were examined by comparing the intake of hay (with or without the fractions) in two-choice test bioassays with cattle. The Fractions A, B and C of the neutral extract stimulated ($p<0.05$) hay consumption in cattle, whereas Fraction D had no effect on selective feeding when compared with the control fraction. Furthermore, Fractions A, B and C had higher ($p<0.05$) feeding stimulative indices (FSI) than that of the Fraction D. The results suggest that chemical stimulants to increase palatability of hay are present in the neutral Fractions A, B and C of high-grain concentrate. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13; No. 2 : 188-191*)

Key Words : Appetite Stimulant, Neutral Extract, Chromatograph, Grain, Cattle, Separation

INTRODUCTION

Cattle, sheep and goats selectively graze pastures and different plants in a pasture are often preferred by each species (Arnold, 1980). Animals are thought to be able to select palatable feeds and reject unpalatable ones in mixed swards and palatability of forage to ruminant animals is generally considered to affect ingestive behavior (Campling, 1970; Kenny and Black, 1984). Domestic and wild herbivores, by means of their olfactory and gustatory senses, may select plant materials based on the presence of either stimulatory or deterrent chemicals within the plants (Müller-Schwarze, 1991). The stimulative chemicals are also considered to induce herbivore livestock to preferentially consume certain foodstuffs. Huston and Van Mourik (1981) observed that sheep had great preference to grains and seeds followed by processed cereals, fruits, vegetables and sweets indicating the possibility of existence of certain chemicals in grains that induce palatability. However, limited studies have been done on the isolation and identification of behaviorally active stimulants from feed palatable to herbivore livestock. In a study with goats and sheep, Dohi and Yamada (1997) demonstrated increased palatability of hay with appetite stimulant ether-soluble acidic substances extracted from a high-grain concentrate. Further studies from this laboratory also indicated that ether-soluble neutral and acid fractions of a compound concentrate diet stimulated hay consumption in cattle (unpublished data). In the present study, we therefore separated chemical substances from ether-soluble neutral extract of the above compound feed using a silica gel chromatograph and tested the stimulative effect of each fraction on hay consumption in cattle.

MATERIALS AND METHODS

Extraction and separation of neutral extracts

The composition of a high-grain compound feed used in the present investigation for extraction and separation of the neutral fraction is given in table 1. Feed sample (800 g) was ground, added to 1000 ml of diethyl ether and then left for 2 hours at room temperature. The ether solution was then filtered, reduced to 150 ml in a draft chamber and stored at -20℃. This was separated into acid, neutral and basic fractions as described by Dohi and Yamada (1997). A total of 4.8 kg feed was extracted and divided into three fractions. The ether-soluble neutral fraction equivalent to 1.6 kg of the feed was diluted to a volume of about 500 ml with n-pentane and then it was concentrated to 100 ml by spraying nitrogen gas at a flow rate of 1 liter per minute in a draft

* Address reprint request to H. Dohi. Tel: +81-8548-2-0144, Fax: +81-8548-2-2280, E-mail: dohi@anim.affrc.go.jp.
[1] Present address: Department of Animal Nutrition, Bangladesh Agriculture University, Mymensingh 2202, Bangladesh.
[a] The authors gratefully acknowledge the financial support given by JST/JISTEC and Grant-in-Aid (Bio Cosmos Program) from the Ministry of Agriculture, Forestry and Fisheries, Japan (BCP 99II-A-1) to conduct this research. The valuable help of Mrs. N. Nakamura and technical assistance of the animal management division staffs at Chugoku National Agricultural Experiment Station during this study are highly appreciated.
Received April 14, 1999; Accepted June 2, 1999

Table 1. Ingredient composition of a high-grain diet

Ingredient	Amount (g/100 g DM)
Flaked rye	47
Flaked corn	30
Wheat bran	10
Soybean meal	9
Molasses-salt and vit-mineral premix[#]	4

[#] Contained vit. A, vit. D_2 and calcium carbonate.

chamber. This procedure was repeated four times and the ether solvent was thus replaced by 100 ml n-pentane. About 10 g sodium thiosulphate was added to n-pentane neutral solution and after 5-6 minutes it was filtered using filter paper (Advantec, No. 5A; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). About 60 g of silica gel (60-200 mesh size for column chromatography, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) in 250 ml n-pentane was transferred to an open column (3 cm i.d.×30 cm height). The height of the silica gel layer was approximately 15 cm and 900 ml of n-pentane was passed through the column to wash the silica gel. The n-pentane solution containing the neutral substances was placed on the top of silica gel. Then the column was developed and elutes were collected with four carrier solutions containing 300 ml of n-pentane (Fraction A), 300 ml of 10% diethyl ether in pentane (Fraction B), 300 ml of 25% diethyl ether in pentane (Fraction C) and then 300 ml of diethyl ether (Fraction D). To separate an ether-soluble neutral fraction equivalent to 4.8 kg of feed, the procedure was repeated three times.

In order to prepare control solvents for each of the above separated fractions, the same four carrier solutions (n-pentane: diethyl ether=100:0; 90:10; 75:25 and 0:100) were poured into the column and elutes were collected, after 100 ml of n-pentane (without neutral substances) was placed onto the column. All the fractions eluted from the silica gel column (neutral and control) were stored at -20℃ until using in the two-choice tests. To examine appetite stimulative response of the four fractions, eight two-choice tests were conducted using cattle with two repeated tests for each of the four fractions.

Two-choice test

The test was conducted in March, 1999 using six adult Japanese Black cattle aged about six years and weighing, on average, 430 kg. They were loosely tied in a house which provided natural day light and ventilation during the experimental period of four successive days. All the animals had previous experience with two-choice test. Except for the duration of the test period, animals were fed 10 kg chopped sorghum silage along with some concentrate daily and allowed to exercise freely for two hours everyday. Water was made available to the animals at all times. The two-choice tests were conducted between 10:00 and 10:30 h and also between 14:00 and 14:30 h in each day. Throughout the duration of the two-choice test, animals were not allowed to consume any other feed during the periods between 10:00 and 14:30 h and also the period between 09:00 and 10:00 h. The cattle were fed 10 kg sorghum silage between 14:30 and 09:00 h of the next morning.

In a two-choice test, twelve troughs (two for each animal) containing 1.0 kg of chopped Italian ryegrass hay treated either with test samples or control solvents were allocated at random to six animals. All the test fractions and control solvents were directly applied to the chopped hay and then mixed properly by hand. The animals were allowed to feed freely from both the troughs and the test was initiated after two preliminary tests. The duration of each test period was 10 minutes and the test was repeated eight times, two tests for each of the four fractions using the same cattle. In each set of two tests for each fraction, position of the feeding troughs (treated and control) were changed from left to right side in order to make even animal's choice to select hay from both of the troughs. Intakes of hay by cattle were recorded at the end of each choice test. A feeding stimulative index (FSI) was calculated using the average intake of two repeated measurements of each animal as Dimock et al. (1991): FSI = (T-C) / (T+C), where T and C were the intake of hay with a test sample and control solvent for 10 minutes, respectively.

Statistical analysis

Data recorded for the average intakes of hay by cattle in two repeated measurements over 10 minutes period, with or without the test fractions, were analyzed by paired t-test to compare significant effects between the neutral fractions and their respective control solvents. However, data for feeding stimulative index were analyzed by two-way ANOVA using SAS (1988) program to identify significant effect of neutral fractions on hay consumption using the statistical model: $Y_{jk}=\mu+T_j+C_k+e_{jk}$, where Y_{jk}=dependent variable, μ=overall mean FSI, T_j=number of test fractions (j=1, 2, 3, 4), C_k= number of cattle (k=1, 2, 3, 4, 5, 6), and e_{jk}=residual error. Duncan's multiple range test at 5% level was done to compare treatment means where F-test was found significant.

RESULTS AND DISCUSSION

In a previous study, Dohi and Yamada (1997)

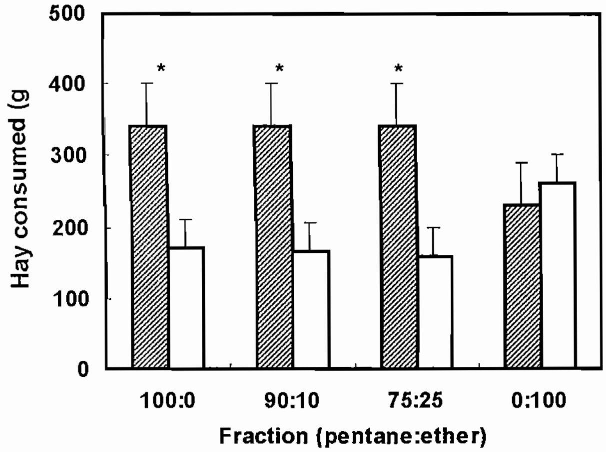

Figure 1. Two-choice tests with four fractions of a high-grain diet separated using an open silica gel chromatograph of ether-soluble neutral extracts. The cross-hatched and open bars depict mean intakes of treated and control hay, respectively, for two repeated measurements using six cattle. Bars represent standard errors, and asterisks indicate significance (*p<0.05; paired t-test).

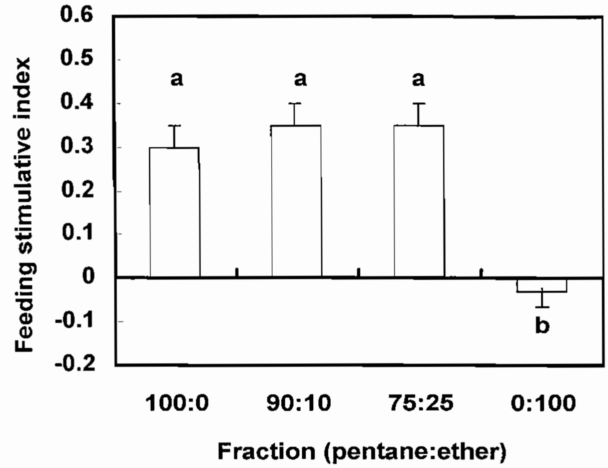

Figure 2. Feeding stimulative responses to the four fractions of a high-grain diet separated using an open silica gel chromatograph of ether-soluble neutral extract. Each column represents the mean feeding stimulative index (FSI) for each treatment. The FSI was calculated using the equation FSI=(T-C)/(T+C), where T and C = the mean intakes of hay with and without a test sample, respectively, for two repeated measurements using six cattle. Columns having dissimilar letters are significantly different ($p<0.05$), and bars denote standard errors.

demonstrated that ether-soluble acid fraction of a high-grain concentrate was attractive to goats and sheep and had a marked influence on hay consumption. However, intake stimulative effects were detected for both of the ether-soluble acid and neutral substances extracted from a high-grain commercial feed in studies with cattle from this laboratory (unpublished data). These results indicated that the feeding preference of herbivore livestock was stimulated by the presence of chemical compounds in feeds. To gain more knowledge about chemical stimuli, the ether-soluble acid fraction of the above high-grain compound feed has been further fractionated into four components and in a preference test with cattle, fraction C eluting with pentane and ether = 75:25 was identified as the greatest stimulative component among the four fractions (data not shown). The present study dealt with the separation of the appetite stimulant ether-soluble neutral extract collected from 4.8 kg of the above feed into four fractions [eluted with pentane and ether=100:0 (Fraction A), 90:10 (Fraction B), 75:25 (Fraction C) and 0:100 (Fraction D)] using an open column of silica gel chromatograph and then tested their stimulative effects on hay intake in cattle.

The average intakes of hay treated with each of the four neutral fractions from a high-grain compound feed and of hay treated only with control solvents are shown in figure 1. When the four fractions at concentrations (50 ml) equivalent to 180 g of concentrate feed were added to Italian ryegrass hay, all the fractions except for the Fraction D stimulated hay intake. The stimulative effects of the Fractions A, B and C on feeding of hay by cattle were significant ($p<0.05$), whereas the Fraction D had no effect when compared with the control fraction. In fact, animals showed almost equal ($p>0.05$) preference for hay treated with control solvent and neutral Fraction D.

The feeding stimulative indices (FSI) of different fractions estimated from the average intakes of two repeated measurements are shown in figure 2. The intake stimulative responses of the animals given the neutral Fractions A, B and C were higher ($p<0.05$) than those that received the Fraction D. However, effects between the Fractions A, B and C were not different. The Fraction C (FSI = 0.35) had a trend to be the greatest stimulative activity followed by the Fractions B (FSI = 0.34) and A (FSI = 0.30) and a negative response was recorded for the Fraction D. In our previous studies, stimulative chemicals of the ether-soluble acidic fraction were separated using the same feed and protocol and then the stimulative effects of these fractions were examined using the same animals as used in the present studies and only the Fraction C produced significant appetite stimulation (unpublished observation). It seems that the stimulative Fractions A and B separated from the neutral extract are different from those in acidic fraction and further separation is necessary to distinguish chemical differences between stimulants of the acidic and neutral Fraction C.

In two-choice tests, cattle preferred the treated hay to control (except Fraction D) after they were repeatedly tested for the Fractions A, B and C. However, it is not clear whether this preference is due to chemical properties of the neutral fractions or to novelty of the treated hay. In general, herbivore animals are unwilling to consume foods they are not accustomed to (Chapple et al., 1987; Burritt and Provenza, 1991; Provenza et al., 1995). For instance, Chapple et al. (1987) reported that it was needed 7 days for sheep to voluntarily consume a novel feed. Therefore, it seems reasonable to suppose that the neutral Fractions A, B and C contain several kinds of chemicals with stimulative activities which were responsible for the preference of the treated hay in the present two-choice tests. The inclusion of the neutral Fraction D decreased hay consumption, indicating that the chemical substances eluted with only diethyl ether did not have appetite stimulative activity as other fractions. Moreover, neophobia resulting from a novel feed may depress feed intake (Dohi et al., 1999).

We hypothesized that the cattle in this experiment had equal frequency to eat hay from both the troughs (right and left). However, we observed that the effect of each fraction varied greatly when it was added to hay placed on the right or left positions of the same animal. Therefore, we measured hay intake from the trough placed on the right and left sides irrespective of treated or control hay. The average 10-minute hay intakes and their standard errors were 0.268 ± 0.022 and 0.220 ± 0.023 kg from the right and left sides, respectively. The animals tended to eat more hay from the right trough, though the difference was not significant. From the results of this study, it is difficult to come to a conclusion and further information using large number of animals is needed to examine the effect of trough position on the ingestive behavior of cattle.

To obtain clear results in intake between treated and control hay, certain points need to be taken into account while conducting preference test. For example, hay after treated with either neutral or control fraction should be left until complete evaporation of all the organic solvents and then offered to animals in order to avoid any influence of the smell of pentane and/or diethyl ether on hay consumption. Moreover, animals should be well acquainted with the test hay and therefore, a longer period of adjustment is necessary before the start of the choice test. Similar quality hay should be provided to the animals and proper mixing of hay with the test fraction may be an important factor in this regard.

Despite the fact, the present study demonstrates that specific appetite stimulants are present in the ether-soluble neutral fraction. It is not clear whether the stimulants might function synergistically or collectively and therefore, we need further separation and identification of the active chemicals.

REFERENCES

Arnold, G. W. 1980. Grazing behavior. In Grazing Animals F. W. H. Morley (Ed.). World Animal Science B1. Elsevier Press, New York.

Burritt, E. A. and F. D. Provenza. 1991. Ability of lambs to learn with a delay between food ingestion and consequences given meals containing novel and familiar foods. Appl. Anim. Behav. Sci. 32:179-189.

Campling, R. C. 1970. Physical regulation of voluntary intake. In Physiology of Digestion and Metabolism in the Ruminant A. T. Phillipson (Eds). Oriel Press, Newcastle, UK. pp. 226-234.

Chapple, R. S., M. Wodzicka-Tomaszewska and J. J. Lynch. 1987. The learning behavior of sheep when introduced to wheat. 1. Wheat acceptance by sheep and the effect of trough familiarity. Appl. Anim. Behav. Sci. 18:157-162.

Dimock, M. B., J. A. A. Renwick, C. D. Radke and K. Sachdev-Gupta. 1991. Chemical constituents of an unacceptable crucifer, *Erysimum cheiranthoides*, deter feeding by Pieris rapae. J. Chem. Ecol. 17:525-533.

Dohi, H., S. Ogura, T. Kosako, Y. Hayashi, A. Yamada and S. Shioya. 1999. Separation of deterrents to ingestive behavior of cattle from cattle feces. J. Anim. Sci. 77:756-761.

Dohi, H. and A. Yamada. 1997. Preference of sheep and goats for extracts from high-grain concentrate. J. Anim. Sci. 75:2073-2077.

Huston, G. D. and S. C. van Mourik. 1981. Food preference of sheep. Aust. J. Expt. Agric. Anim. Husb. 21:575-582.

Kenny, P. A. and J. L. Black. 1984. Factors affecting diet selection by sheep. 1. Potential intake rate and acceptability of feed. Aust. J. Agric. Res. 35:551-563.

Müller-Schwarze, D. 1991. The chemical ecology of ungulates. Appl. Anim. Behav. Sci. 29:389-402.

Provenza, F. D., J. J. Lynch and C. D. Cheney. 1995. Effects of a flavour and food restriction on the response of sheep to novel foods. Appl. Anim. Behav. Sci. 43:83-93.

SAS. 1988. SAS/STAT@ User's Guide (Release 6.03). SAS Ins. Inc., Cary, NC.