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ORIGINAL RESEARCH PAPER

Estimation of pasture herbage intake by beef cattle based on *n*-alkanes as markers

Piotr Nowakowski, Katarzyna Czyz*, Marta Iwaszkiewicz

Institute of Animal Breeding, Faculty of Animal Science, Wrocław University of Environmental and Life Sciences, Chełmońskiego 38c, 51-630 Wrocław, Poland

* Corresponding author. Email: katarzyna.czyz@interia.eu

Abstract

The aim of the study was to evaluate herbage dry matter intake in 16 beef cows which grazed continuously on permanent pastures within the "The Warta Estuary" National Park (Poland), using the minimally invasive method based on *n*-alkanes as markers (C_{29}, C_{31}, C_{33}). Significant differences were observed in the nutritive value of herbage collected for analyses by cutting or nipping. The calculated content of energy and protein in the nipped herbage was higher: UFL by 58.1% and PDI by 50%, with a higher digestibility of nutrients. The values obtained for DM intake in pasture herbage by cows were closest to the standards when calculations were based on the C_{29}/C_{32} pair of *n*-alkanes. However, the best prediction of DM intake estimation from creeping bentgrass (*Agrostis stolonifera*) pasture, in agreement with the accepted energy and protein standards, was based on the proportions between alkanes C_{31}/C_{32}.

Keywords

cattle; grass intake; dry matter; *n*-alkanes

Introduction

The estimation of pasture herbage intake can be based on direct or indirect methods. The direct method consists of weighing animals before and after grazing, determining the quantity of feed consumed and feces by weight [1]. Another method is based on the use of a rising plate meter (RPM) for a determination of the height and weight of the herbage on pasture [2]. In the indirect method, the estimation of herbage intake is based on collection of excreted feces after an indigestible marker is added to the feed or directly to the rumen. Such a marker should easily mix with the feed, pass through the digestive tract at the same rate as the feed, and be easy to analyze. The natural indicators occurring in feeds or added to the diet (orally or through a fistula) include lignin, chromogene stains, SiO_2, including acid-insoluble ash (AIA), indigestible acid detergent fiber (IADF), or long-chain *n*-alkanes. Also, chemical indicators can be added to the diet, e.g. Cr_2O_3, Cr marked fiber, Cr-EDTA, Co-EDTA, polyethylene glycol (PEG), elements such as La, Yb, and Ce [3].

Oesophagus fistulas were used in order to estimate the consumption of herbage dry matter by grazing ruminants [4]. Currently, for ethical reasons, alternative estimation methods are being explored. One has been described by Mayes et al. [5] and consists of a determination of the level of *n*-alkanes in the herbage and feces. Alkanes are compounds not digested by ruminants and naturally occurring in plants in form of chains with an odd number of carbons (from C_{25} to C_{35}). Introducing alkanes with an even number of carbon atoms directly into the rumen, with simultaneous collection of feces and herbage samples, renders it possible to determine the consumption of dry matter and to estimate its digestibility [5,6]. The *n*-alkane indicator method of estimating the intake of herbage by cows from pasture proved to be more useful than other methods [1]; using this method, Reeves et al. [2] and Estermann et al. [7] obtained precise estimations of the intake of dry matter from pastures. Malossini et al. [8] considered the *n*-alkane method as simpler than the chromium oxide method. Similarly, Smit et al. [1] as well as Reeves et al. [2] stated that the *n*-alkane method is the best for determining the pasture dry matter intake. Vulich et al. [9] described its repeatability for the estimation of feed consumption by animals as high, while Estermann et al. [7] reported that optimum results were obtained when the samples were collected in the morning hours.

The *n*-alkane indicator may be used not only for estimating the consumption of pasture herbage but also of additional intake of other feeds, as for instance additional herbage or silage [10]. Work was conducted on the simplification of the determination of *n*-alkanes and reducing work expenditure and costs [4,11]. Hendricksen et al. [12] used molasses as a carrier for the alkane markers C_{32} and C_{36}, assuming that it will have no negative effects if offered three or five times per day.

The work aimed at estimating the intake of herbage dry matter using the indicator method and at determining the differences in nutrient intake arising from the use of different alkanes as markers (C_{29}, C_{31}, C_{33}) in cows grazing permanent pastures in the protected area. Grazing the meadows and grasslands in the protected areas is a significant issue in terms of biodiversity and ecological balance maintenance. Animal welfare, in turn, is one of especially important issues in such areas, and therefore minimally invasive methods are the most desirable in case of any procedures related to the animals inhabiting these areas.

Material and methods

The experiment was conducted on a natural pasture within the "The Warta Estuary" National Park from mid-August to the end of the first week of September 2005 (22 days in total). The cattle herd grazed continuously on a paddock marked as "Bridge IV", about 300 hectares in area, located between the rivers Warta and Postomia; the animals were kept free without buildings. Botanical analysis of the pasture herbage was performed before the start of the experiment, and it demonstrated that the dominating species was creeping bentgrass (*Agrostis stolonifera*) – an 86.2% share of plants in total herbage mass.

During the experimental period, the weather was sunny (no precipitation), the temperature during the day was at a level from 9–11°C (morning) to 18–20°C (midday). The mornings were misty and the pasture herbage was covered with dew from sunrise until the late afternoon. The animals had constant access to a natural watering place (Postomia River).

The animal material comprised 16 cows of three breeds: Hereford (5), Simmental (7), and Limousine (4). All the cows were rearing calves in the second month of lactation and were selected from a herd consisting of 340 cows with calves. The live body weight of the cows ranged from 403 to 711 kg. The experimental animals were weighed, their condition was estimated according to a 5-point score (body condition score – BCS), and they were marked by identification collars.

Pasture herbage height (cm) and dry matter content were determined at the beginning and end of the experiment (in kg DM/ha). After the first (initial) 7 days following the introduction of internal markers, feces samples were collected individually for each animal, directly after defecation, once per day between 7 and 10 a.m., and they were used to prepare pooled samples.

A Captec Alkane CRC bolus (Captec Ltd., New Zealand), containing an external marker (8 g ditriacontane, C_{32} and 8 g hexatriacontane, C_{36}), was introduced once (using an applicator) through the oesophagus into the rumen. According to the producer's information, the marker should be released into the rumen over a period of 20 days. Alkane C_{32} was treated as the basic marker, while alkane C_{36} constituted a control for the bolus and simultaneously was a control for quantitative determinations of C_{32}. The manipulation was performed in a cattle crush, i.e., a metal construction enabling immobilization of the animal during the treatment.

Feces samples were collected until Day 22 of the experiment in order to determine precisely the total duration of alkane release and next the input into the rumen over 24 hours. Simultaneously, two pooled herbage samples were obtained: one from herbage cut at a height of 3.0 cm (±0.5 cm) from the pasture surface and the second one nipped from locations visited by the experimental animals and at a height at which the cows grazed, i.e., about 10 cm above the ground. The collected herbage and feces samples were dried and then subjected to a basic chemical analysis according to the guidelines contained in [13] in order to determine the nutritive value of the feed.

Basic chemical composition of the pasture herbage (fat, protein, fiber, ash, acid detergent fiber – ADF, natural detergent fiber – NDF) was determined using standard analytical methods [14]. The content of *n*-alkanes (C_{29}, C_{31}, C_{32}, C_{33}, C_{34}, C_{36}) was determined in the feces and herbage with the use of a gas chromatograph after fat extraction from the samples, according to the method described by Mayes et al. [5].

The dried samples of herbage and feces (1 g DM) were subjected to 15-hour extraction with an addition of 0.6 mg C_{34} (internal standard) in a Soxhlet apparatus with ethyl ether. The extract was transferred quantitatively into a reaction vessel and vaporized dry in a mild nitrogen stream. The dry sample, after an addition of 2 cm^3 benzene and 2 cm^3 BF_3 (20% solution in methanol), was heated for 4 hours at a temperature of 90°C. After cooling and adding 4 cm^3 hexane and 1 cm^3 water, the content was shaken for 15 minutes; next, the exsiccated layer was separated and vaporized dry. The rest was dissolved in 1.5 cm^3 hexane, transferred onto a chromatograph column filled with 5 cm^3 gel 60 (70–230 mesh), and eluted with 10 cm^3 hexane. The eluate was vaporized dry and next dissolved in 0.1 cm^3 hexane.

The separation of alkanes was performed on a gas chromatograph (INCO type 505) in the following conditions: the filling consisted of 1% Dexil 400 GC on Chromosorb W-HP, 80–100; FID detector temperature = 290°C, temperature of initial column = 150°C for 10 min, and next the +6°C/min temperature program was applied; after reaching a temperature of 320°C, it was retained for 20 minutes. Identification of the peaks obtained as a result of the chromatographic separation of alkanes occurring in the feces and herbage was performed by comparing the relative retention periods obtained (in relation to C_{34}) with the relative retention periods obtained from an analysis of alkane standards (C_{29}, C_{31}, C_{32}, C_{33}, C_{36}; Sigma-Aldrich). The concentration of *n*-alkanes in the pasture herbage and feces dry matter was corrected (correction indicator) for the level of *n*-alkanes recovered during the chromatography analysis (Tab. 1).

The following measures and indicators were used in the present work:

- The production of feces was calculated on the basis of the C_{32} external indicator, according to the following formula: *feces production* (kg DM/day) = D_j / (K_j – R_j)
- The dry matter digestibility was calculated on the basis of the concentration of the indicator C_{31} in the herbage and feces, according to the formula: *dry matter digestibility* (%) = 100 – (100 × R_i / K_i)
- The consumption of herbage dry matter was calculated on the basis of the C_{29}, C_{31}, C_{33} to C_{32} proportions in the herbage and feces, according to the formula presented by Mayes et al. [5]: *dry mater intake* (kg/day) = (K_i D_j / K_j) / (R_i – K_i R_j / K_j)

where: K_i and R_i are the respective concentrations (mg/kg DM) of natural alkanes with odd numbers of carbon (internal indicators, C_{29}, C_{31}, or C_{33}) in the feces and herbage; K_j, R_j, and D_j are the respective quantities of alkanes with even numbers of carbon (external indicator, C_{32}) in the feces and herbage (mg/kg DM/day) and in a daily oral dose (mg/day).

The results obtained for the herbage dry matter intake by animals, expressed in kg/day and g/kg of metabolic body weight ($W^{0.75}$/day), were compared with standards. The estimated intake of herbage DM according to C_{29}/C_{32}, C_{31}/C_{32}, C_{33}/C_{32} was compared with the intake of nutrients – energy and protein.

The final selection of a pair of alkanes (C_{29}/C_{32}, C_{31}/C_{32}, C_{33}/C_{32}) for estimating DM consumption from creeping bentgrass (*Agrostis stolonifera*) was based on a comparison of the results obtained for DM, energy and protein intake with standards [13–16].

Tab. 1 Recovery of alkane standards from the chromatography column and the correction indicator.

Item		Alkanes					
		C_{29}	C_{31}	C_{32}	C_{33}	C_{34}	C_{36}
Recovery of 1 mg alkane from the chromatography column	x	0.894	0.94	0.03	0.893	0.906	0.917
	SD	0.0094	0.094	0.133	0.0179	0.0123	0.0282
	V%	1.1	1.1	1.0	2.0	1.4	3.1
Correction indicator (*r*)		0.940	0.940	0.903	0.893	0.906	0.917

Results

It was estimated that 3000 ±800 kg herbage DM per ha was present on a pasture not grazed by animals (height 12.7 ±4.3 cm), while after grazing only 1900 ±500 kg DM/ha (height 8.7 ±2.4 cm). The grazing pressure was estimated as a mean of 0.88 animal units per unit of herbage mass.

In the samples of herbage cut and nipped, the content of ash, crude fiber, fat, and ADF was similar, but a higher content of protein and a lower content of NDF were detected in the nipped herbage samples (Tab. 2). The nipped herbage samples were a more accurate estimation of herbage consumed by ruminants than the cut herbage samples that also contained lignified shoots and dead plant parts.

The mean live body weight of cows was 582.9 ±83.13 kg, while the estimated body condition score was 3.8 ±0.49 BCS in a 5-point scale.

On average, the production of feces amounted 2.8 ±0.5 kg DM per day. The highest digestibility (%) of herbage DM was obtained when calculations were based on alkane C_{31} (56 ±6.1), and alkane C_{29} (54 ±6.7), while calculations based on alkane C_{33} resulted in the lowest digestibility of DM (41 ±9.3).

The important step in the experiment was to determine the actual time of alkane release from the boluses compared with the times given by the producer (Captec). Based on the actual release of C_{32} and C_{36} alkanes checked in the feces on Day 18 to 22, it was determined that the introduced alkanes were voided from the boluses for 20 days, i.e., in accordance with the information of the producer. The variation in the total release time of 8 g of C_{32} alkane (example: 20, 21, and 22 days) relates to the level of its daily release into the rumen (i.e.: 400 mg/day, 381 mg/day, 364 mg/day).

The data presented in Tab. 3 concerning dry matter intake by the examined breeds of cows based on the C_{29}, C_{31}, and C_{33} alkane content in pasture herbage and the external marker C_{32} showed no statistically confirmed differences.

The intake of nutrients presented in Tab. 4 demonstrates significant differentiation between the nipped and cut herbage. The values calculated were significantly lower for cut herbage, both in case of UFL and PDI, and for C_{29}/C_{32} and C_{31}/C_{32} as a reference of DM intake.

Limousine cows, heavier than Herefords (by about 100 kg), consumed less herbage DM calculated for the metabolic body weight (g/kg $W^{0.75}$) and also less energy and protein (Tab. 5). The coefficient of correlation between the live body weight of cows and herbage DM intake, calculated for metabolic body weight g/kg $W^{0.75}$, proved to be negative and highly significant ($r = -0.90$; $p \leq 0.01$) in the present study.

Tab. 2 The chemical composition (mean ±*SD*) and nutritive value of 1 kg DM of cut and nipped herbage.

Herbage	Cut	Nipped
Content in dry matter		
Ash	9.60 ±0.586	10.17 ±0.559
Crude protein	16.96 [a] ±1.187	23.72 [a] ±1.898
Crude fibre	20.18 ±1.816	20.18 ±1.816
Fat	2.19 ±0.153	2.34 ±0.171
Natural detergent fibre	54.12 [a] ±2.706	48.93 [a] ±2.349
Acid detergent fibre	22.61 ±1.357	21.39 ±1.390
Nutritive value*		
UFL	0.74	1.17
UFV	0.60	1.14
PDIN (g)	94	128
PDIE (g)	74	111
PDI (g)	74	111

a – means marked with the same superscript differ at $p \leq 0.05$.
*According to INRA standards [13].

Tab. 3 Estimation of dry matter intake (kg) of different breeds of cows on the basis of the content of alkanes C_{29}, C_{31}, C_{33} in pasture herbage and the external marker C_{32}.

Genotype	Hereford	Simmental	Limousine	Mean
Mean body weight (kg)	551.0 ±88.77	556.1 ±80.78	669.5 ±65.29	582.9 ±83.13
Intake based on C_{29} / C_{32}	10.63 ±0.55	10.92 ±0.61	11.14 ±0.40	10.89 ±0.55
Intake based on C_{31} / C_{32}	6.95 ±0.25	7.10 ±0.32	7.18 ±0.19	7.07 ±0.27
Intake based on C_{33} / C_{32}	7.05 ±0.19	6.75 ±0.24	6.95 ±0.38	6.89 ±0.28
According to Vladiveloo and Holmes [16]	9.98 ±0.79	11.65 ±0.58	6.31 ±0.34	11.42 ±1.25
According to traditional standards [15]	11.76 ±0.73	13.26 ±0.37	14.02 ±0.24	12.78 ±1.02

Tab. 4 The intake of nutrients (UFL, PDI*) by cows calculated on the basis of DM estimate from the proportion of alkanes C_{29}/C_{32} and C_{31}/C_{32} and the chemical composition of nipped (n) and cut herbage (c).

	C_{29}/C_{32} as reference of DM intake				C_{31}/C_{32} as reference of DM intake			
	UFL		PDI (g)		UFL		PDI (g)	
Genotype	n	c	n	c	n	c	n	c
Hereford	12.39 ±0.941	7.84 ±0.591	1175.7 ±89.04	783.8 ±59.36	8.06 ±0.185	5.10 ±0.110	763.6 ±19.77	509.0 ±13.19
Simmental	12.80 ±0.387	8.09 ±0.246	1214.6 ±36.79	809.8 ±24.53	8.37 ±0.333	5.30 ±0.200	794.5 ±31.32	529.7 ±20.89
Limousine	13.04 ±0.470	8.25 ±0.298	1237.8 ±44.58	825.2 ±29.71	8.37 ±0.192	5.30 ±0.122	795.7 ±19.15	530.5 ±12.74
Mean	12.74 ^{A} ±0.626	8.05 ^{B} ±0.395	1208.3 ^{A} ±59.29	805.5 ^{B} ±39.52	8.27 ^{A} ±0.309	5.24 ^{B} ±0.189	785.1 ^{A} ±30.19	523.4 ^{B} ±20.13

The calculations were based on mean results obtained from pooled, 6-day samples of feces and herbage. $^{A,\,B}$ – means marked by different superscripts – difference significant at $p \leq 0.01$. *According to INRA standards [13].

Tab. 5 Comparison of the genotypes of cows in relation to DM, energy, and protein intake per metabolic body weight ($W^{0.75}$), estimated on the basis of the proportion of alkanes C_{31}/C_{32}.

				Energy intake / kg $W^{0.75}$		Protein intake g / kg $W^{0.75}$	
Genotype	N	Body weight (kg)	DM g/kg $W^{0.75}$	n	c	n	c
Hereford	5	551 ±89	58.6 ±8.62	0.072 ±0.01	0.046 ±0.006	6.82 ±0.97	4.55 ±0.65
Simmental	7	556.1 ±87	60.3 ±6.99	0.074 ±0.008	0.047 ±0.005	7.03 ±0.81	4.69 ±0.54
Limousine	4	669.5 ±65	52 ±3.91	0.063 ±0.005	0.040 ±0.003	6.07 ±0.44	4.05 ±0.29
Mean	16	583 ±93	57.68 ±7.38	0.070 ±0.009	0.045 ±0.005	6.73 ±0.84	4.48 ±0.29
Significance of genotype differences ($p \leq$ value)		0.091	0.190	0.180	0.197	0.193	0.190

n – nipped; c – cut herbage.

Cows with a higher live body weight consumed more herbage DM, energy, and protein when calculated in direct values: $0.255 \le r \le 0.340$ ($p \le 0.05$). Herbage DM intake (kg) was significantly correlated with the consumption of energy and protein (UFL and PDI; g), irrespectively of the accepted nutritive value of herbage collected by nipping or cutting ($0.620 \le r \le 0.663$; $p \le 0.05$).

Discussion

The condition of cows determined in the study was average; it should be remembered that cows being in a poorer condition tend to consume more herbage DM [15] – the degree of the animal's adiposity affects its energy requirements and thus also its ability to consume DM in feed offered ad libitum.

The theoretical intake of pasture herbage DM was calculated from the ratio of different alkanes with odd numbers of carbon atoms (C_{29}, C_{31}, C_{33}) to the internal marker C_{32} (Tab. 3). Hereford, Simmental, and Limousine cattle had no statistically confirmed differences in the intake of DM. The estimation of herbage DM intake by cows was most similar to standard values [16,17] when the calculations were based on *n*-alkanes C_{29}/C_{32}. Calculations of DM intake based on the ratio of *n*-alkanes C_{31}/C_{32} and C_{33}/C_{32} resulted in values lower by 54.0 and 58.5%, respectively, when the release of the external marker C_{32} was 400 mg/day (Tab. 3).

The intake of herbage by a suckler cow in lactation, calculated from the ratio between alkanes C_{31}/C_{32} and C_{33}/C_{32}, remained at a similar level of about 7 kg DM/day. These data differ considerably from the accepted physiological standards [13,15–17], in which this value ranges from 9 to 14 kg DM/day. In the case of herbage dominated by creeping bentgrass, the estimation of DM intake by cattle calculated from the C_{31}/C_{32} and C_{33}/C_{32} ratios was clearly too low. In turn, when the calculations were based on alkane C_{29}, the results obtained were close to the physiological standards. The results reported by other authors recommend the use of various pairs of alkanes for the estimation of DM intake by cows. For instance, Smit et al. [1] as well as Vulich et al. [9] obtained results of high precision using alkanes C_{33}/C_{32}, while Estermann et al. [7] alkanes C_{31}/C_{32}. Reeves et al. [2] recommend that the estimation of DM intake from a pasture dominated by *Pennisetum clandestinum* should be based on a ratio of alkanes C_{33}/C_{32}. Similar results of DM intake were obtained by Nia and Wittenberg [18] when the authors determined the quantity of herbage consumed by ruminants using either C_{31}/C_{32} or C_{33}/C_{32}.

Differences were observed in the herbage intake calculated on the basis of pooled and individual samples; e.g., the DM intake calculated on the basis of an individual sample obtained from one cow (collar No. 22) was by about 30% higher, and for another cow (collar No. 04) by about 20% lower than that obtained on the basis of pooled samples. This finding is not consistent with the results reported by Vulich et al. [9] who showed that there were no statistically significant differences in estimations of DM intake based on individual or pooled samples.

The quantity of consumed energy and protein was calculated in relation to the DM estimated, and a comparison was made between the intake of nutrients in cut (c) and nipped herbage (n) (Tab. 4).

When the reference alkane contained C_{29}, the estimated DM intake were in agreement with the standard values. The calculated intake of protein and energy (PDI and UFL) was also close to standards if the calculations were based on the chemical composition of cut herbage. However, the composition of nipped herbage was more similar to the herbage consumed by grazing ruminants. When its nutritive value was used for calculations based on the C_{29}/C_{32} alkane pair, the estimated intake of protein and energy proved to be considerably higher than the accepted physiological standards.

The use of the reference alkanes C_{29} and C_{31} resulted in clearly different values of DM intake by animals. When the reference alkane contained C_{29}, the estimated DM intake remained in agreement with standards [13,15,16], but when the reference alkane contained C_{31}, the estimated DM intake values were clearly lower (on an average by 35%). When the calculations were based on the chemical composition of nipped herbage and alkane C_{29}, the energy and protein consumption was overestimated compared to that accepted in the requirement standards.

Conclusions

Statistically significant differences were observed in the estimation of the nutritive value of herbage collected for analyses by cutting or nipping. The nipped herbage contained 28.5% more protein and 9.8% less fiber (NDF by 10.6% and ADF by 5.7%) and as result the calculated content of energy and protein was higher: UFL by 58.1% and PDI by 50%, with a higher digestibility of nutrients.

The estimated values obtained for herbage DM intake by cows were closest to the norms when calculations were based on the C_{29}/C_{32} pair of *n*-alkanes; the estimations of DM intake based on the proportions of *n*-alkanes C_{31}/C_{32} and C_{33}/C_{32} were lower by 54.0 and 58.5%, respectively, than the standard values reported in the literature. However, the best prediction of DM intake estimation from creeping bentgrass pasture, in agreement with the energy and protein norms accepted, may be obtained when the calculations are based on the proportions between alkanes C_{31}/C_{32}.

It must also be remembered that the nutrient requirements of cattle differ between DLG [13], INRA [14], and IZ [15] standards – animals of the same live body weight and the same physiological status are shown to have different requirements for energy and protein. This may be due to the fact that these standards are developed in various countries and can be presumably based on local breeds of cattle and also fodder as well as climatic conditions generating various nutritional needs.

Cows with a higher live body weight consumed more DM, energy, and protein in the herbage (in direct values); however, heavier cows (Limousine) consumed less herbage DM when calculated for metabolic body weight (g/kg $W^{0.75}$) compared to cows of breeds characterized by lower body weights: Hereford and Simmental (by 11.1% and 14.3%, respectively).

The present work shows that the estimation of forage consumption by cows grazing permanent pastures should be based on samples containing nipped herbage.

Summarizing, one may state that the indicator *n*-alkane method, applied to beef cows grazing in a large herd in open areas of a National Park, rendered possible precise estimation of herbage DM intake as well as of the consumption of energy and protein without disturbing the animals' welfare.

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Ocena spożycia runi łąkowej przez bydło mięsne w oparciu o zawartość wskaźnikowych *n*-alkanów

Streszczenie

Celem badań była ocena spożycia suchej masy roślinnej na przykładzie 16 krów mięsnych, które w sposób ciągły wypasane były na pastwiskach w Parku Narodowym „Ujście Warty" (Polska), z zastosowaniem minimalnie inwazyjnej metody wykorzystującej jako wskaźniki *n*-alkany (C_{29}, C_{31}, C_{33}). Istotne różnice zaobserwowano w przypadku wartości odżywczej roślinności pozyskanej do analizy przez koszenie lub uszczykiwanie. Obliczona zawartość energii i białka była wyższa w przypadku roślin uszczykiwanych: UFL o 58.1%, a PDI o 50%, czemu towarzyszyła wyższa strawność składników odżywczych. Wartości dotyczące pobrania suchej masy wraz z roślinnością przez zwierzęta były najbliższe norm, gdy obliczenia oparto na stosunku *n*-alkanów C_{29}/C_{32}. Najdokładniejsze oszacowanie pobrania suchej masy z mietlicy rozłogowej (*Agrostis stolonifera*) z pastwiska, zgodne z przyjętymi normami dotyczącymi energii i białka, uzyskano w oparciu o stosunek alkanów C_{31}/C_{32}.