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Annual Fat Cycle of Field Mice,
Apodemus flavicollis (Melchior, 1834)*

[With 1 Fig. & 4 Tables]

The fatness in yellow-necked field mice, *Apodemus flavicollis* (Melchior, 1834) ($N = 95$) from beech forests near Cracov was examined in a complete annual cycle (from winter to winter). Fat content has been determined in Soxhlet's apparatus by ether extraction. The mean biomass of animals examined during various seasons amounted to 26.3 ± 6.20 g, while the mean dry matter — to 7.9 ± 1.84 g. Water content was highest in summer (72.8%), and lowest in winter (on an average 68.3%). Fat content in mouse bodies reveals seasonal fluctuations and a distinct annual cycle. Since autumn mice accumulate fat (on an average $13.9\% \pm 3.54$ of the dry body weight); in winter the fat content is highest ($20.8\% \pm 5.13$). In spring fat amount drops quite obviously ($11.9\% \pm 6.11$) and in summer it is maintained on the same low level ($11.9\% \pm 3.31$). The fatness of winter mice differs statistically significantly from that in all remaining seasons. Females have somewhat higher fat content than males, also adult mice contain more fat than juvenile ones. Caloric value of mouse fat reaches 9.124 — 9.305 kcal/g, while that of fat-free tissue — 4.209 — 4.782 kcal/g. Hence the calculated energy value of the rodent body is higher in winter than in summer (by 5% when compared with the ash-free dry weight, and 23% in relation to biomass).

I. INTRODUCTION

Energy value of animal bodies (*i.e.* their net production) fluctuates in various groups within a very broad range, when expressed in kcal/g of dry weight (Golley, 1959; 1961). These values, when calculated in relation to ash-free dry weight, are more compact. In invertebrates, representing distant taxonomic group, caloric value of body is included within the range from 5.4 to 6.4 kcal/g of ash-free dry weight (Slobodkin, 1962; Slobodkin & Richman, 1961). In terrestrial mammals this value may fluctuate from 4.5—8.0 kcal/g (Górecki, 1965, Golley, 1967). Highest values may be expected only seasonally in hibernating mammals or migrating birds (Odum *et al.*, 1965).

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Górecki (1965) found very significant seasonal changes in caloric value of body in non-hibernating small mammals, connected with seasonal accumulation of fat in bodies of these animals. Animal fats are highly caloric substances and their energy value often exceeds 9.0 kcal/g (Kleiber, 1961). Hence a slight change in animal fatness may be responsible for the considerable change in caloric value of its body (Górecki, 1965). So far informations concerning fat content in bodies of small mammals are rather scarce and they only occasionally refer to annual cycles (Connell, after Golley, 1962).

The present paper describes seasonal changes of fatness in yellow-necked field mice, *Apodemus flavicollis* (Melchior, 1834) in a complete annual cycle. The yellow-necked field mouse is one of the two dominating rodent species in the forest ecosystems of central Europe. This is why it has been selected for studies under the Polish Small Mammal Project of IBP.

II. MATERIAL AND METHOD

All yellow-necked field mice came from the Ojców National Park in the vicinity of Cracow (50°13'N, 19°40'E), where they were trapped mainly in beech forests (*Fagetum carpaticum*). In the course of one and a half year mice were trapped in six periods: autumn of 1965 (October), winter of 1966 (February), spring of 1966 (April—May), summer of 1966 (July), autumn of 1966 (October), and winter of 1967 (February). During each season the fat content has been determined in a series consisting of 12—20 animals, altogether of 95 mice (Table 1).

Table 1.

The numbers of field mice which were studied in all seasons.

Season	Number of animals	♂♂ + ♀♀	Body weight in g.	
			Mean ± S.D.	C.v.
Autumn 1965	12	5+7	28.29 ± 5.30	18.0
Winter 1966	13	5+8	28.19 ± 6.46	22.2
Spring 1966	20	13+7	28.75 ± 5.96	20.7
Summer 1966	17	9+8	24.10 ± 8.32	34.5
Autumn 1966	18	9+9	24.34 ± 7.09	29.1
Winter 1967	15	5+10	23.92 ± 3.55	14.8
Total average	95	46 49	26.26 ± 6.20	23.6

Yellow-necked field mice, trapped in snap traps, were measured, their sex and age were determined, and they were weighed with the accuracy to the nearest 0.01 g. Mean body weight for all material amounted to 26.26 g (Table 1). Isolated stomach content was also weighed, since the mice were extracted without it.

Technique of preparing the mice for extraction consists in crushing and possibly quick drying the animal body. For this purpose a mouse cut into small pieces was being placed in an extraction thimble made of ash-free paper filter. Mouse body together with thimble was then dried to the constant weight in vacuum oven at the temperature of +60°C. That drying lasted no longer than three days. Vacuum oven considerably shortens the time of drying and, besides, enables drying at lower temperature which prevents the decomposition of fats (Górecki, 1965a).

Before extraction the dried sample was placed for 30 minutes into anhydrous ethyl alcohol to denature proteins. That prevented their leaching together with fat during the extraction of sample. Alcohol was vaporized from beaker, while the remaining fat was still added to that obtained in extraction (Forbes, 1966). Extraction was carried out with ether in Soxhlet apparatus (Odum *et al.*, 1965) in bulbs with the volume of 250 or 500 ml, placed in water bath with the temperature of 52–55°C. One animal was extracted on an average 25 times, *i.e.* during 8–10 hours. The extraction was done in three series with 10 + 10 + 5 runs, changing ether after each series. Following to extraction the sample has been dried to constant weight (in 60–70°C) and accurately weighed. Maximum number of extractions was 42 times for a sample, while the minimum one — 14 times. The longest was always the first series of extraction, because then about 80% of the total fat amount was leached.

Ether extract has been vaporized in weighing bottles in the drier with forced air circulation at 37°C. Ether was completely vaporized from the sample after about 48 hours. After each extraction the quantity of extracted fat was checked and compared with the fat calculated from the difference between the dry weight of animal and the fat-free weight. Generally, extraction yielded slightly less fat than the calculation. The difference resulted from losses connected with the volatilization of certain amount of fat together with rapidly vaporizing ether, but never exceeded 3%.

In the last winter series (1967) there was carried out additional procedural experiment, in which for 7 animals the fat contained in skin and that contained in carcass were extracted separately.

In both winter seasons and in summer the fat and fat-free weight remaining after extraction were burned in a calorimeter bomb according to the procedure described by Górecki (1965a,b). The ash content in fat-free weight has been also determined in an electric muffle oven.

From measurements the water content and fat per cent in the body of mouse were determined. In summer and in both winters ash percent and caloric value of dry weight were determined, also the caloric value of ash-free weight and caloric value of biomass have been calculated.

III. RESULTS

All data for subsequent seasons represent mean values for series consisting of 12–20 individuals (Table 1, 2). Although the numerical ratio of males to females was somewhat different in subsequent series, the sex ratio for all the material was almost as 1:1 (Table 1).

Mean biomass of mice in various seasons of the year was similar and fluctuated within limits from 23.9 g up to 28.7 g (Table 1). In autumn of 1965, winter and spring of 1966 the examined animals were heavier (on an average by 28.4 g). Coefficients of body weight variation are rather low for all these seasons and on an average reach 20% (Table 1). During three following seasons (summer and autumn of 1966 and winter of 1967) the mean biomass of mice was slightly lower and amounted to 24.1 g. In summer and autumn this was connected with proportion

of juvenile animals in samples (cf. the coefficient of variation for these seasons — Table 1). On the other hand, the lower weight of mice in winter of 1967, when compared with the previous winter, may indicate the lower condition of animals during that period.

The mean dry weight for whole material at all seasons is contained within limits from 6.5 up to 9.3 g (Table 2). It reaches its highest value during winter and the lowest one — during summer, its variation (C.v. = 13.9 — 35.8%) and seasonal fluctuations are almost identical with biomass variation (Table 1). The per cent of water was highest in summer — 72.8%, while in spring and autumn it was somewhat lower (by some 2.2%). The animals had the lowest water content in winter, on an average in both winters they contained 68.3% of water (Table 2).

The curve of fatness in yellow-necked field mice during the studied period indicates two distinct peaks — in both winter seasons. In an annual cycle one can note that animals collect fat since autumn and have

Table 2.
Seasonal changes of fat content and water content in the body of field mice.

Season	Dry wt. of body in g	Water content (%)	Fat per cent of dry weight	
	Mean±S.D.	Mean±S.D.	Mean±S.D.	C.v.
Autumn 1965	8.39±.41	70.9±1.57	13.2±2.86	22.0
Winter 1966	9.33±2.18	66.8±1.89	20.8±5.13	24.6
Spring 1966	8.71±1.94	69.8±2.20	11.9±6.11	51.3
Summer 1966	6.53±2.34	72.8±1.41	11.9±3.31	27.8
Autumn 1966	7.03±2.04	71.0±1.94	14.5±4.23	29.2
Winter 1967	7.18±1.00	69.9±1.50	16.7±4.21	25.2
Average	7.86±1.84	70.2±1.78	14.8±4.41	30.0

it most in winter; in spring its value drops very abruptly and in summer it is maintained on the same low level (Fig. 1). During the winter of 1966 mice had the maximum fat content (20.8% of body dry weight), while the minimum one during the spring and summer of 1966 (11.9%). The mean fat content in animal body during both autumns reached 13.9%. During the winter of 1967 mice were slightly less fatty than in the preceding winter, however the per cent of fat when compared with remaining seasons is still high and amounts to 16.7% (Table 2, Fig. 1). The coefficient of variation was exceptionally high in the spring series, in remaining seasons it is by far lower (Table 2).

Statistical analysis of seasonal changes in fat content in the bodies of mice reveals that differences between the winter of 1966 and all the remaining seasons are very highly significant ($P < 0.001$). Both winters differ from each other at the level of high significance

($P < 0.01$). When the winter of 1967 is compared with the remaining seasons it differs with very high statistical difference from summer, and with high significance from the autumn of 1965 and spring 1966. Summer differs significantly from the autumn of 1966 ($P < 0.05$). There are no statistical differences among all remaining seasons.

There were found certain differences in the fatness of males and females. The mean fat content in carcasses of all examined females amounted for all seasons to 15.2% and, except spring, was higher than in males (14.2%). In spring males had more fat in their bodies (12.8%

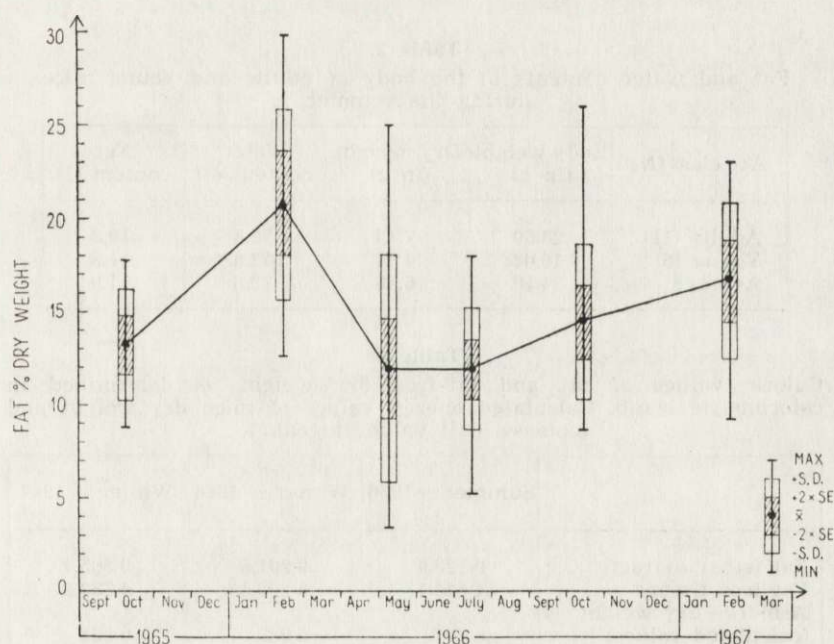


Fig. 1. Seasonal changes in fat content of yellow-necked field mouse body during the period of years 1965—1967. The curve (solid line) joins mean values for all seasons. The range of variation is shown by vertical lines (max.-min.). The white rectangle represents one standard deviation (SD) on each side of the mean. The blackened portion of the rectangle comprises two standard errors (SE) of the mean on each side of \bar{x} .

and 10.4%, respectively). That was connected probably with the beginning of breeding period in these animals. If fat per cent calculation were done without spring, then for females it would reach on an average 16.2%, while for males only — 14.5%. These values do not differ statistically.

The mice examined during summer were classified as adult and juvenile, since the material during that season was highly differentiated

in respect to age (11 adults and 6 juveniles). The biomass and dry weight of juveniles are almost by half lower than in adults. Water content was identical in both groups, but fat per cent in juveniles is only slightly lower (Table 3). This difference, although physiologically justified, is not significant statistically.

The extraction of fat from carcass and skin revealed that the amount of fat in carcass presents $16.1 \pm 4.42\%$, while in skin — $18.1 \pm 6.31\%$. There are also no statistical differences between these values.

Caloric values were determined only in summer and in both winters. Winter fat has slightly higher caloric value than the summer one. This

Table 3.

Fat and water contents in the body of adults and young mice during the summer.

Age class (N)	Body weight (in g)	Dry weight (in g)	Water content (%)	Fat content (%)
Adults (11)	28.50	7.73	72.8	12.3
Young (6)	16.02	4.34	72.8	11.3
Average	24.10	6.53	72.8	11.9

Table 4.

Caloric values of fat and fat-free dry weight as determined in calorimetric bomb. Calculated energy values of mice dry weight and biomass. (All values in cal/g).

	Summer — 1966	Winter — 1966	Winter — 1967
Fat (ether extract)	9 123.9	9 201.5	9 305.1
Fat-free tissues	4 544.1	4 209.4	4 782.2
Ash-free dry weight (calculated values)	5 817	5 937	6 291
Fresh weight (biomass) (calculated values)	1 379	1 736	1 661

value is very similar for both winters: on an average it amounts to 9.253 cal/g and exceeds the caloric value of summer fat by only 129 cal/g (Table 4). Energy value for whole animal was calculated with the aid of determinations of caloric values for fat and fat-free weight, as well as fat and ash content in mouse body (Table 4). Thus calculated energy value of ash-free dry weight is somewhat higher in winter. The mean for both winters exceeds data for summer only by 5%. On the other hand, the caloric value of biomass, calculated from the caloric value of dry weight and water content, is higher in winter than in summer by 23% (Table 4).

IV. DISCUSSION

Ether was selected for fat extraction on the basis of experiments by research workers, who during recent years had determined the fatness of mammals and birds (Odum, 1960; Odum *et al.*, 1965; Wolff, 1963; Jameson & Mead, 1964; Kale, 1965; Forbes, 1966). Other authors also used successfully fat solvents such as: benzene — alcohol at 4:1 ratio (Kreiner, 1958), ethyl alcohol — ether at 3:1 ratio, methanol — chloroform at 1:2 ratio, ethyl alcohol — ether (Sperry, 1955), benzene alone (Sealander, 1951), toluene (Wolff & Bakay, 1963), ether — alcohol (Connell *et al.*, 1960). In the course of pilot experiments for the present work several of these solvents were tried and it was found that ether is the best and most convenient, mainly owing to its low boiling point (34.6°C).

As a result of ether extraction one obtains »ether extract« called also sometimes »crude fat«. It consists mainly of true fats, but contains also free fatty acids, cholesterol, sterols, mono, di-, and triglycerides, phospholipides, vitamins, and dyes. Usually only some of them and at minimal quantities occur in animal tissues. The concept of ether extract is already commonly accepted in bioenergetic literature both by nutrition physiologists (Kleiber, 1961) and by ecologists (Odum, 1960; Kale, 1965).

Informations about body fatness in small rodents are rather scarce. Different authors relate seasonal changes in fatness with such factors as: environmental temperature (Sealander, 1951), natural food supply (Connell, after Golley, 1962), physiological status and age of animals (Jameson & Mead, 1964), and even changing population density (Hsia-Wu-ping & Sun-Chung-lu, 1963). Fat content in rodent bodies is characterized also by a considerable individual and geographical variation (Hayward, 1965).

The annual cycle of fatness has a different course in hibernating when compared with non-hibernating mammals. In not hibernating rodents the annual amplitude of changes in fatness is considerably smaller than in hibernators. *Peromyscus leucopus* has the highest fat content in winter and this is why these animals are heavier at this time (Sealander, 1951). In various subspecies of *Peromyscus maniculatus*, acclimated to laboratory conditions, fat content was also higher in winter, than in summer (Hayward, 1965). During winter wild rats also increase considerably the quantity of fat in their bodies (Héroux, 1961). The curve of fatness for *Peromyscus polionotus* has two peaks throughout the year, one in winter and another in summer (Connell, after Golley, 1962). In *Clethrionomys rutilus* from

China the highest relative fatness was recorded during spring, time while the lowest one in summer (Hsia-Wu-ping & Sun-Chung-lu, 1963). Quoted examples indicate that in non-hibernating rodents of temperate zone one can expect the highest fatness during winter. The yellow-necked field mouse finelly confirms this rule. This is probably rather common adaptation of small rodents for winter in temperate climate. For reserves provide the better isolation properties of body and also present a valuable energetic reserve.

In literature not many numerical values determine accurately the fatness in rodents. For laboratory mice Wolff & Bakay (1963) gave the mean per cent of fat — 15.6. From Connell's graph (Golley, 1962) one can note in *Peromyscus polionotus* that per cent fluctuates from approximately 15 up to 27. The level of fat content found in yellow necked field mouse (11.9—20.8%) is thus very similar to these data.

The present paper was intended to answer the question if seasonal fluctuations in the caloric value of rodent body could be explained by a change in fat content in these animals (Górecki, 1965). For the yellow-necked field mouse from Ojców, Górecki (1965) determined the caloric value of ash-free dry weight on 6086 cal/g during summer, and 5045 cal/g at the end of winter and during early spring. The values calculated in Table 4 are very similar during summer (5817 cal/g), but distinctly higher during winter (on an average 6114 cal/g). This comparison is not complete, however, due to two reasons. Our winter mice were captured in February, while in Górecki's paper (1965) the winter and early spring series contained mice trapped from March until the end of April. It results from cited data that the fatness of mice is lowest just in April and May (Table 2). Besides, in Ojców beech forests there occurred a heavy crop of beechmast in 1964, while during years 1965—1966 trees had almost no seeds. Mice population from late summer of 1964 until the spring of 1965 fed at Ojców mainly on highly caloric beechmast (Drożdż, 1966). Population density of mice not especially high in 1964, was rapidly increased and reached maximum during the summer of 1965, and afterwards dropped abruptly. In the further course of the spring of 1966 mice numbers started gradually to rebuild (Grodziński *et al.*, 1966; Bobek *in litt.*). And so mice from the summer and autumn of 1964 (Górecki, 1965) came from the increasing population, while mice population during the summer of 1966 started only to grow from a decline in numbers and could not feed on rich in fat beechmast.

The caloric value of rodent bodies closely corresponds with fat content in body of these animals. The fatness alone appeared to be

a very sensitive index of population processes and population trophic situation. Since fluctuations in woodland rodent numbers occur at several years long cycle, so seasonal changes in fatness and energetic value of their bodies may have also somewhat different course in various years. For the estimate of net production of rodent population one should use rather the caloric value of body, average for various seasons of year (G ó r e c k i, 1965).

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SEZONOWE ZMIANY OTŁUSZCZENIA U MYSZY LEŚNYCH
APODEMUS FLAVICOLLIS (MELCHIOR, 1834)

Streszczenie

U myszy leśnych, *Apodemus flavicollis* (Melchior, 1834) zbadano sezonowe zmiany otłuszczenia ciała w pełnym cyklu rocznym. Zwierzęta do określeń pochodziły z lasów bukowych Ojcowskiego Parku Narodowego koło Krakowa. W ciągu półtora roku złowiono łącznie 95 myszy, które pochodziły z sześciu okresów: jesień 1965, zima, wiosna, lato i jesień 1966, oraz zima 1967 (Tabela 1). Zawartość tłuszczu w ciele myszy leśnych określano ekstrahując go eterem w aparacie Soxhlet'a. W obu sezonach zimowych i w lecie spalono w bombie kalorymetrycznej

tłuszcz i odtłuszczonej masę pozostałą po ekstrakcji. W piecu do spalań oznaczano jeszcze zawartość popiołu w masie odtłuszczonej.

Srednia biomasa zwierząt dla wszystkich sezonów wynosiła $26,3 \text{ g} \pm 6,20$ (Tabela 1). Srednia sucha masa dla całego materiału wynosiła $7,9 \text{ g} \pm 1,84$. Procent wody był największy w lecie ($72,8\%$), a najniższy w zimie (średnio $68,3\%$) (Tabela 2).

Zawartość tłuszczu w ciele myszy zmienia się sezonowo i posiada wyraźny cykl roczny. Od jesieni myszy leśne gromadzą tłuszcz (średnio $13,9\% \pm 3,54$ suchej masy ciała) i w zimie mają go najwięcej ($20,8\% \pm 5,13$). Na wiosnę ilość tłuszczu spada bardzo wyraźnie ($11,9\% \pm 6,11$) i w lecie utrzymuje się na takim samym niskim poziomie ($11,9\% \pm 3,31$) (Tabela 2, Ryc. 1). Otłuszczenie myszy zimowych różni się istotnie statystycznie od otłuszczenia we wszystkich pozostałych sezonach. Samice są nieco bardziej otłuszczone niż samce. Myszy dorosłe zawierają więcej tłuszczu (średnio $12,3\%$) niż młode ($11,3\%$) (Tabela 3).

Wartość kaloryczna tłuszczu myszy wynosi $9,124\text{--}9,305 \text{ kcal/g}$, podczas gdy tkanki odtłuszczonej $4,209\text{--}4,782 \text{ kcal/g}$ (Tabela 4). Wyliczona stąd wartość energetyczna ciała gryzoni jest wyższa w zimie niż w lecie (o 5% w stosunku do suchej masy bez popiołu, a o 23% w stosunku do biomasy).

Wartość kaloryczna ciała gryzoni koresponduje ściśle z zawartością tłuszczu w ich ciele. Samo otłuszczenie okazało się bardzo czułym wskaźnikiem procesów populacyjnych i sytuacji pokarmowej populacji. Ponieważ fluktuacje liczebności gryzoni leśnych odbywają się w cyklu kilkuletnim, dlatego sezonowe zmiany otłuszczenia i wartości energetycznej ich ciała mogą mieć także nieco odmienny przebieg w kolejnych latach.