Определение географического места происхождения, аутентичности и нелегального использования гормонов в животноводстве

методом масс-спектрометрии стабильных изотопов

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- Пестициды
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- Лекарственные средства
- Радиация
- Микробиология

- **Место происхождения**
- **Стероидные гормоны**
- **Соответствие заявленной «органичности» продукции**

ISOSTER PROJECT

Determination of the origin of hormones in cattle (ISOSTER)

General Project Information

FP5 Programme Acronym: ***GROWTH***

Project Reference: G6RD-CT-2001-00616
Contract Type: Cost-sharing contracts
Start Date: 2002-01-01
End Date: 2005-12-31
Duration: 48 months
Project Status: Execution
Project Acronym: **ISOSTER**
Update Date: 2005-05-11

Project Description

The use of steroid hormones to enhance growth in animal production is banned in the EU since 1988. Hormones used to promote growth in livestock farming, are known to be related to cancer in laboratory animals and humans. The Council Directive 96/23/EC regulates the surveillance of these substances and residues in the animals and animal products. Due to this ban there is a necessity of developing a system including analytical methods for monitoring the presence or the concentrations of anabolic steroids in offal and excrements of animals reared for meat production. The ISOSTER project is aiming at developing and validating a Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry technique for official control purposes of illegal administration of natural hormones. The aim is to be able to use the method for not only the control of urine but also for other matrices like faces, muscle, kidney, liver, blood and fat.

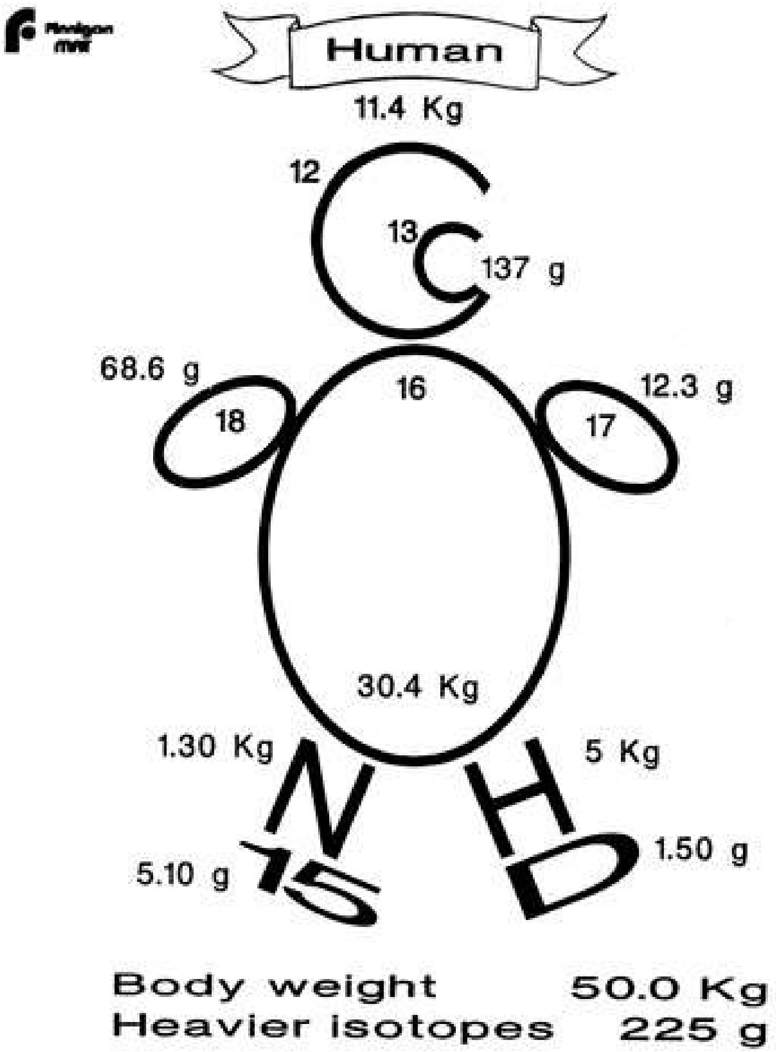
Информация IRMS

- Точные изотопные отношения

Element	**Minor Isotope**	**Natural abundance [%]**
Hydrogen	^{2}H (D)	0.015**57**
Carbon	^{13}C	1.111**40**
Nitrogen	^{15}N	0.366**30**
Oxygen	^{18}O	0.200**04**
Sulfur	^{34}S	4.215**00**
others		

That‘s where the information is

“Изотопный человек”



Почему масс-спектрометрия стабильных изотопов?

- Изотопные отношения изменяются
  Они изменяются под действием следующих факторов:
  - Радиоактивные распад – показывает время
  - Изменение фазового состояния
    - испарение / конденсация
    - плавление / кристаллизация
  - Химические реакции
  - Фракционирование под действием внешних сил
  - Искусственное обогащение/обеднение

Изотопные отношения водорода и кислорода на Земле

Physical Fractionation of Isotope Ratios

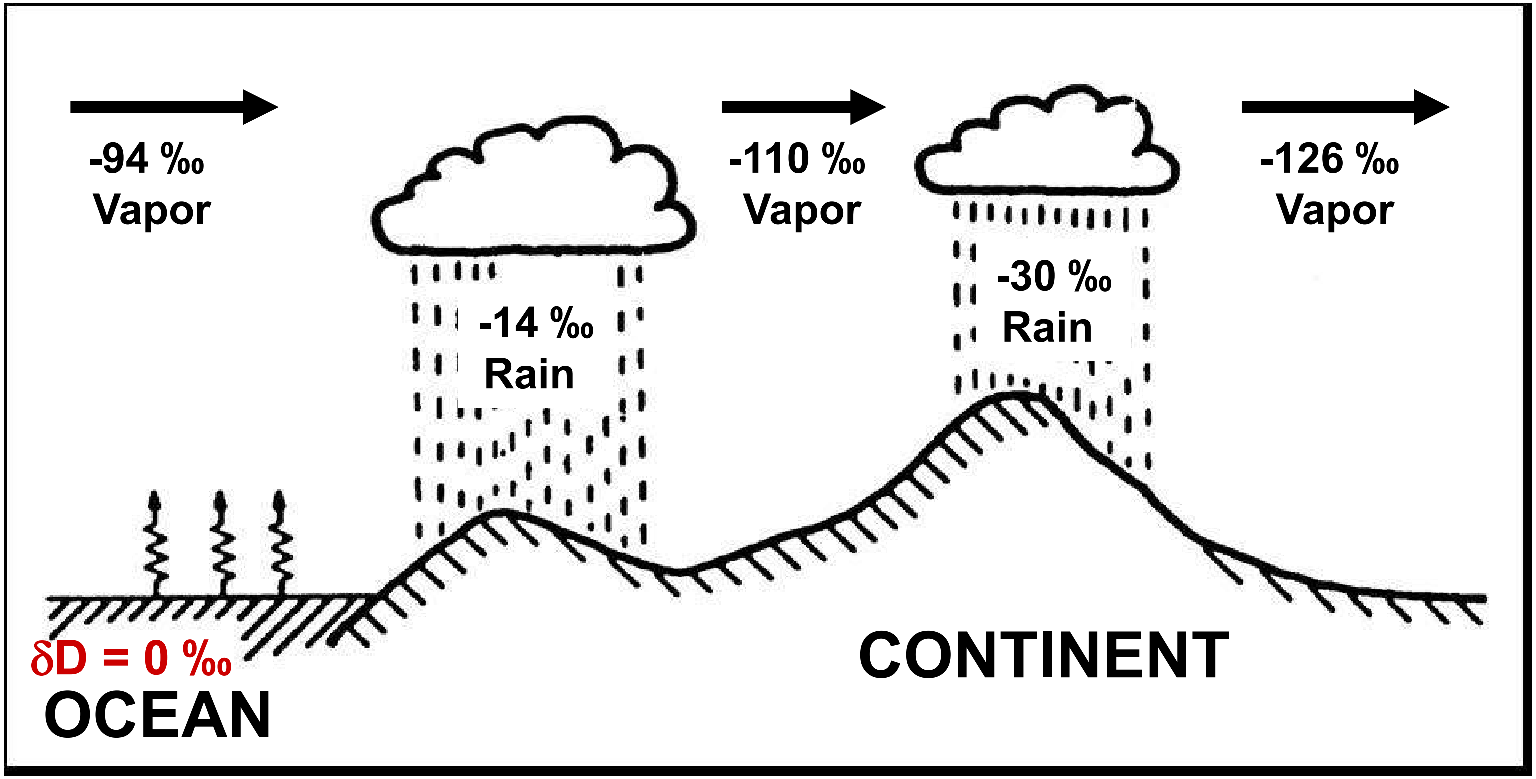
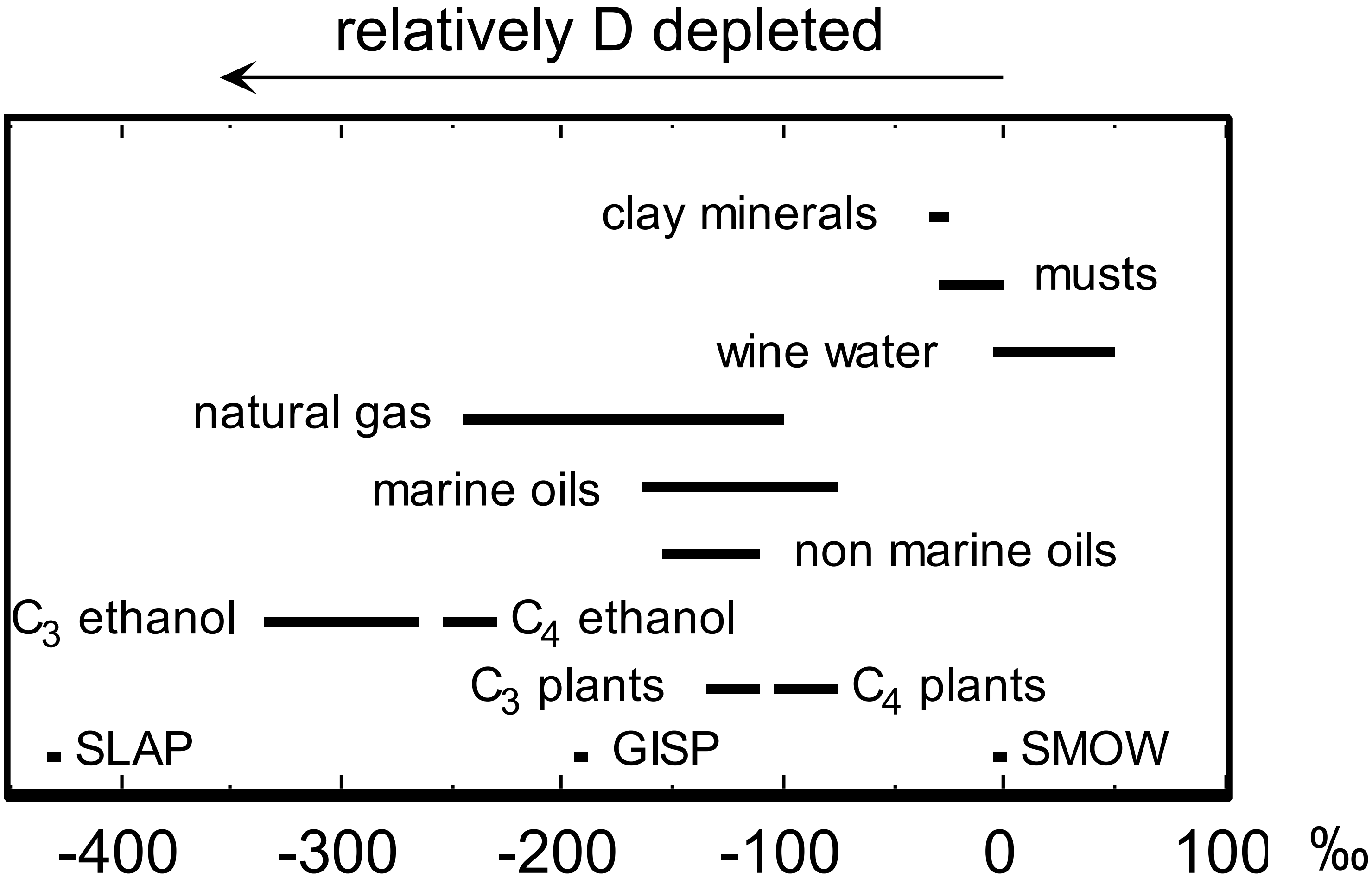


Схема фракционирования в атмосферном водяном цикле

Значения δ^2H для различных сред



Стратегия масс-спектрометрии стабильных изотопов

Как работает изотопная масс-спектрометрия

- Преобразование в простые газы, например CO_2
- Специальный источник ионов (EI, gas tight)
- Многоколлекторная система детектирования на коллекторах Фарадея
  - *Одновременное детектирование, например m/z 44, 45, 46*
- Определение изотопных отношений
- Савнение образца / стандарта

H_2
N_2
CO
O_2
CO_2
SO_2

Пробоподготовка и преобразование в простые газы

- Окисление
- Сжигание
- Пиролиз
- Фторирование
- Химическое окисление
- Восстановление
- Уравновешивание
- Биохимические реакции
- Газовая хроматография
- Жидкостная хроматография

Устройства пробоподготовки и интерфейсы

H_2
N_2
CO
O_2
CO_2
SO_2

The Delta V Series

- World Wide Success
  - Based on over 900 Delta IRMS sold in over 70 countries
- The most sensitive Delta IRMS ever
  - DELTA V Plus: Less than **800** Molecules to form a CO_2 Ion
- A Break-Through Analyzer Concept
  - Monolithic Analyzer with Intrinsic Alignment of all Ion Optical Parts
- One Concept – Two Isotope Ratio Engines
  - Full Upgradeability from DELTA V Advantage to DELTA V Plus
  - HD on-line for both Engines
- High Productivity Turn-Key System
  - Rugged, Reliable and Easy-to-use

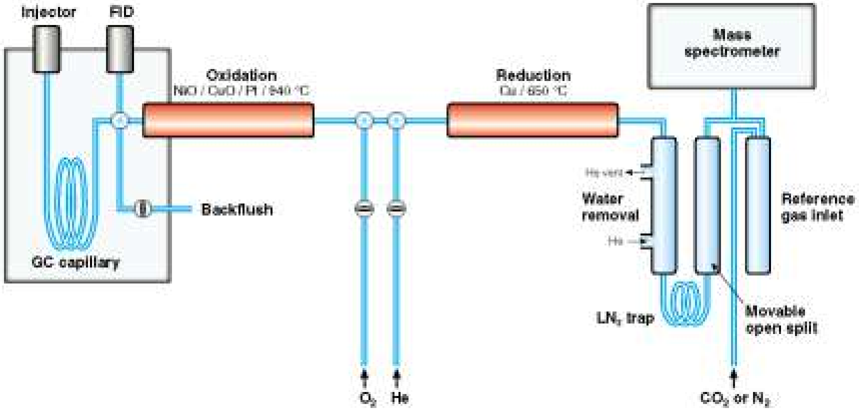
DELTA V Advantage

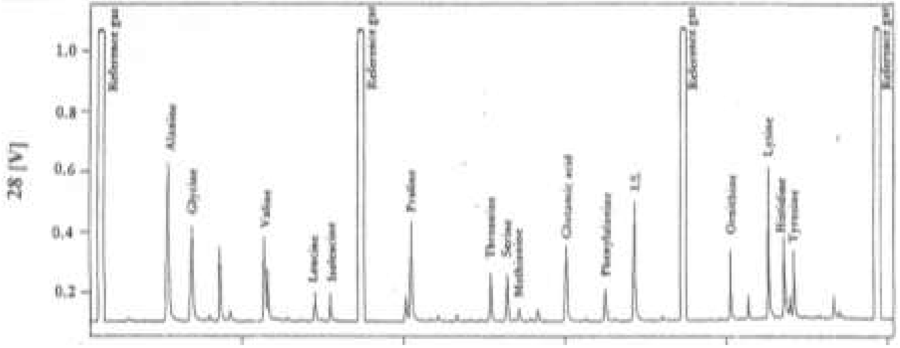
Flash EA HT

AS 3000, MAS200R

Газовая хроматография – изотопная масс-спектрометрия

- GC-C/TC III Interface for ^{13}C, ^{2}H, ^{15}N, ^{18}O

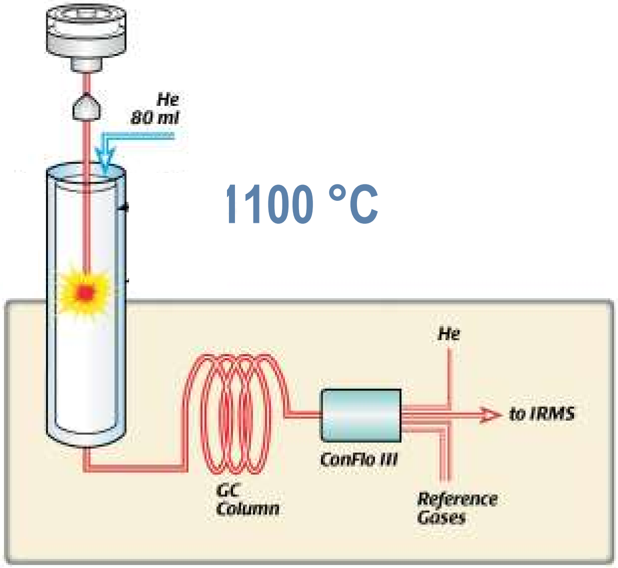




Amino Acids, O-iPropyl, N-Pivaloyl Derivatives

Элементный анализ – изотопная масс-спектрометрия

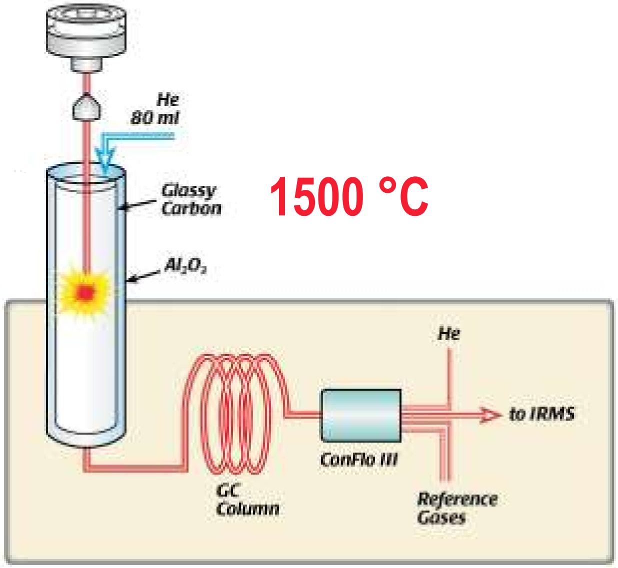
- Flash Combustion



1100 °C

$\delta^{13}C$ & $\delta^{15}N$
$\delta^{34}S$

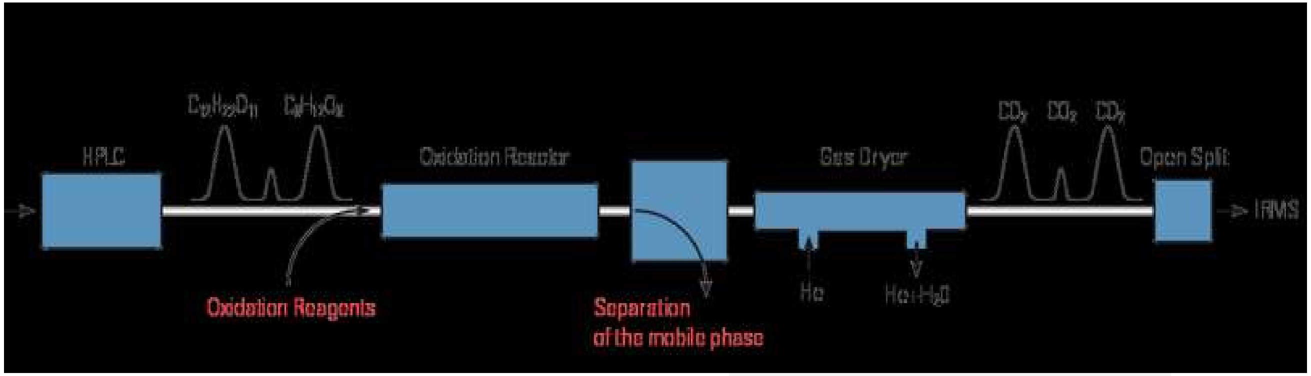
- High Temperature Conversion

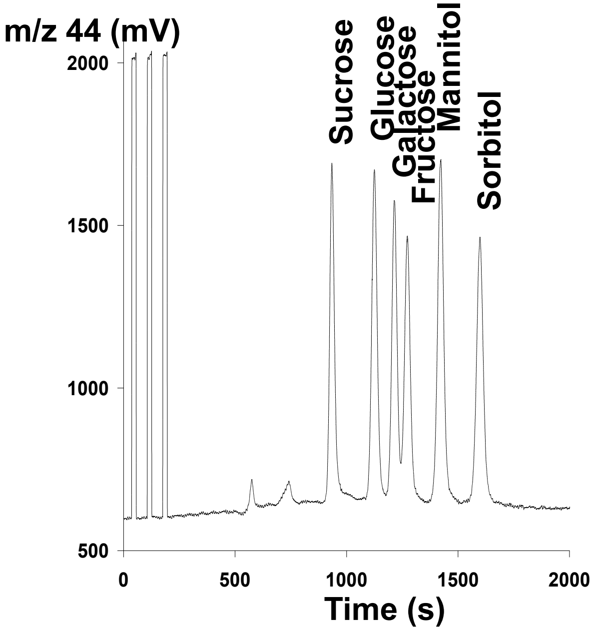


1500 °C

$\delta^{2}H$ & $\delta^{18}O$

ВЭЖХ – изотопная масс-спектрометрия





DELTA V Plus

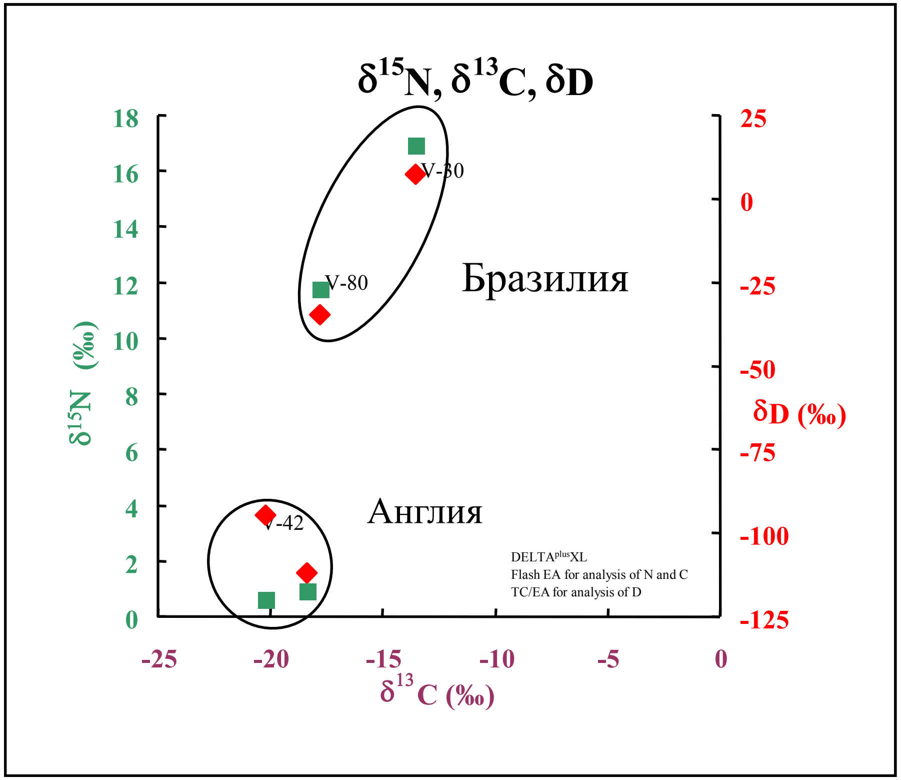
LC IsoLink

Surveyor HPLC System

Определение географического происхождения говядины

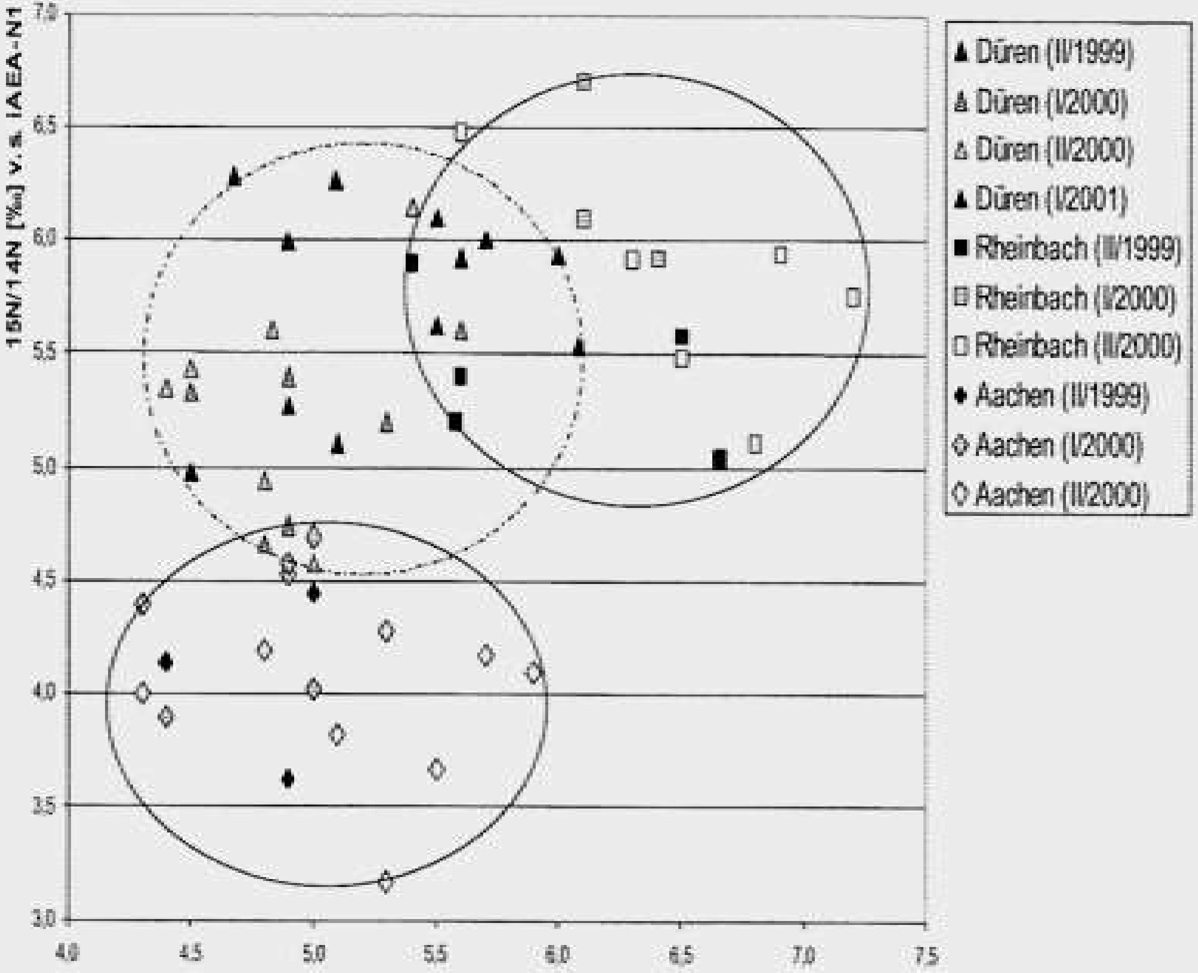
IRMS^3
TC/EA
& EA

Изотопный анализ обезжиренной сухой массы

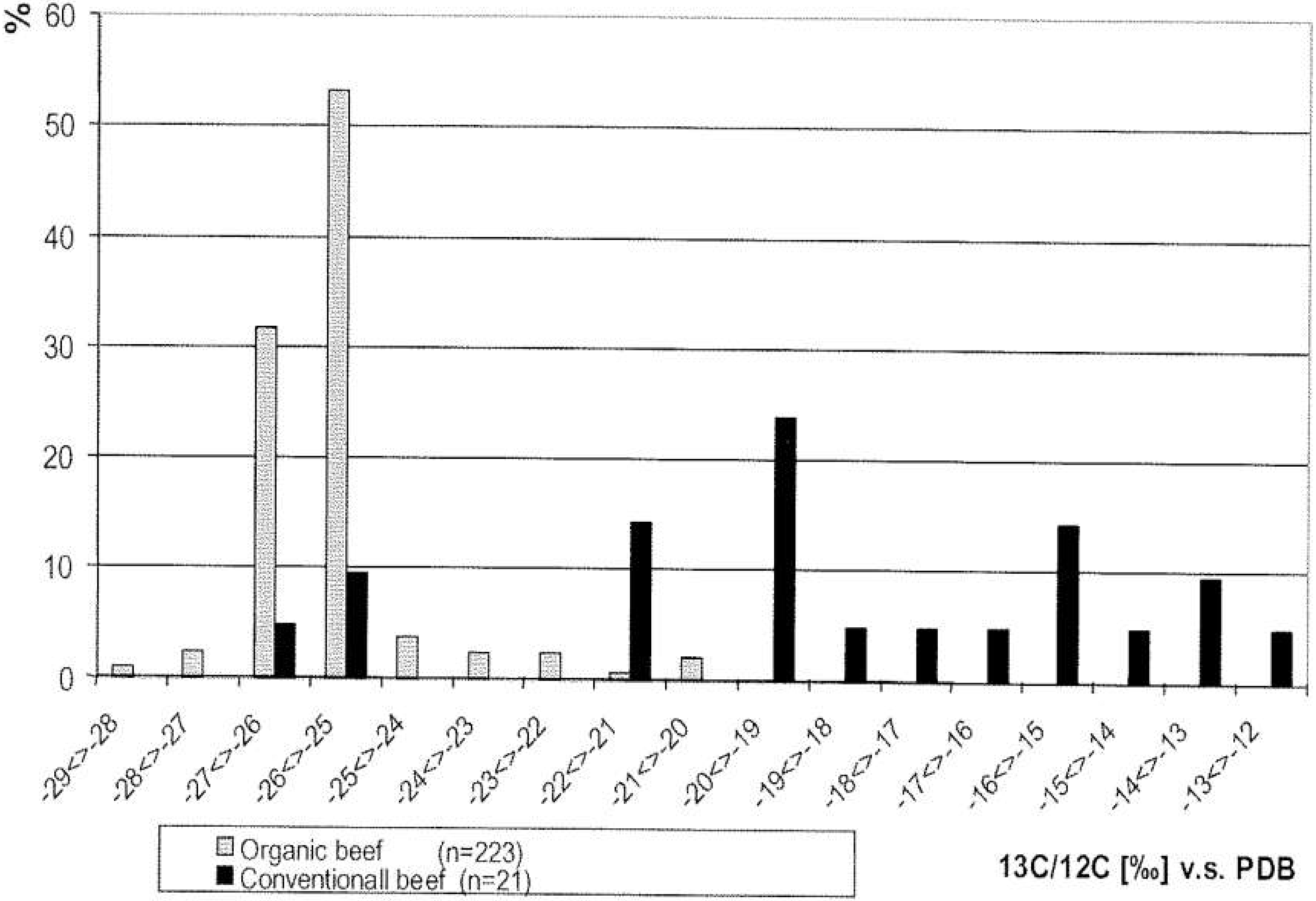


$^{15}N/^{14}N$ и $^{34}S/^{32}S$ для говядины из различных ферм

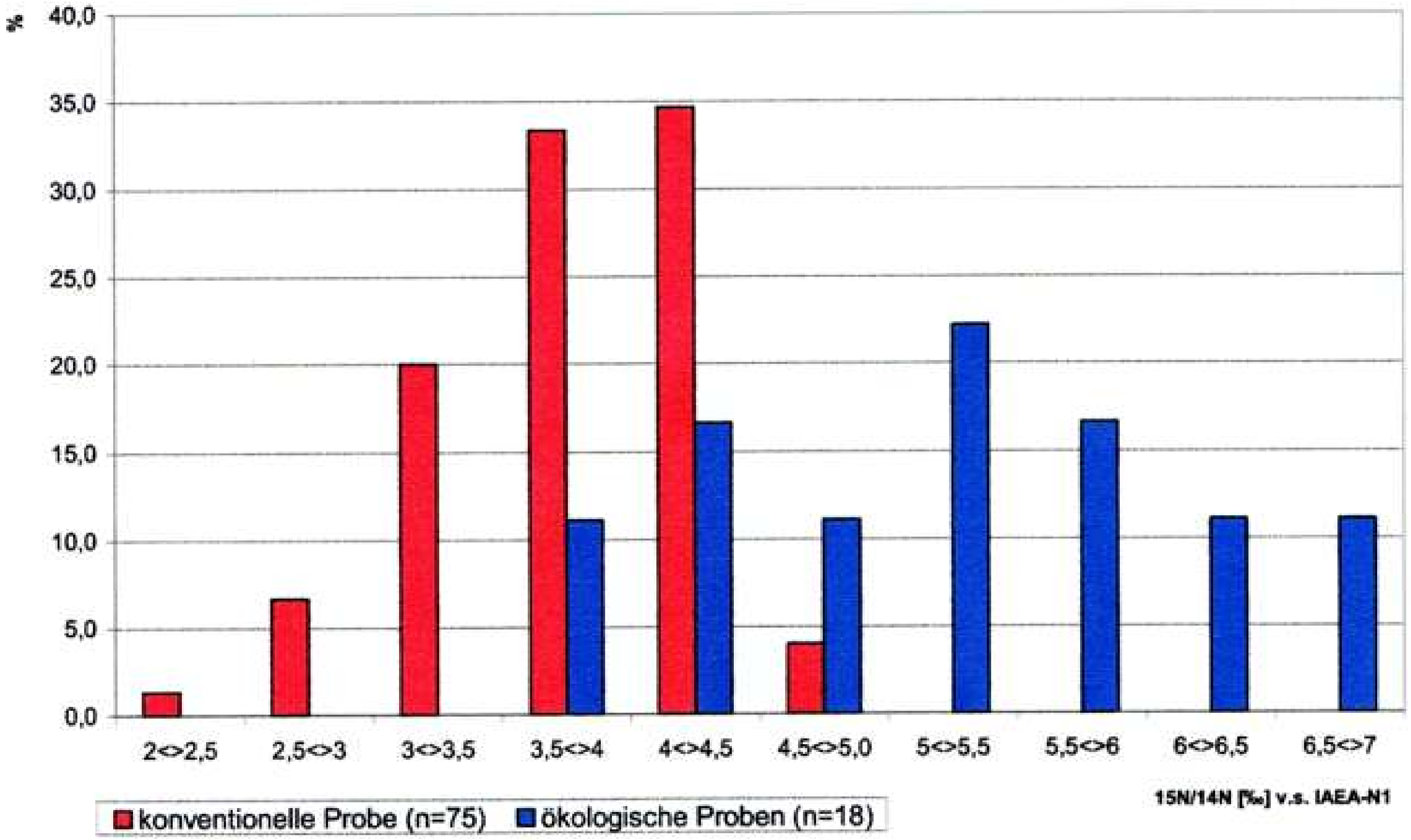
Fig. 5 Local geographical pattern of the stable isotopes nitrogen and sulfur: *I* Jan–Jun, and *II* Jul–Dec. The samples belong to the three farms: Aachen, Dueren and Rheinbach



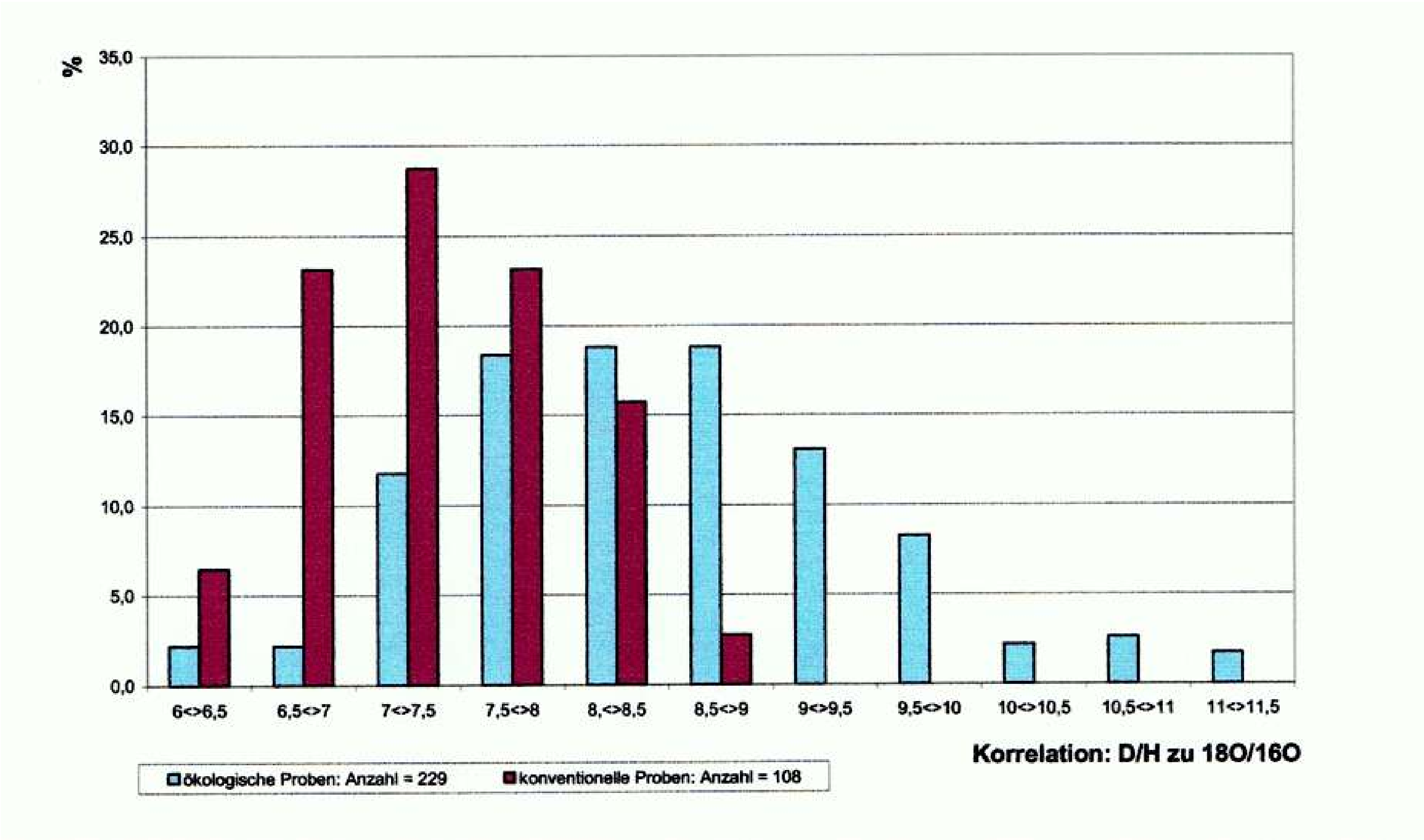
$^{13}C/^{12}C$ для органической и обычной говядины



$^{15}N/^{14}N$ для обычных и «органических» яиц

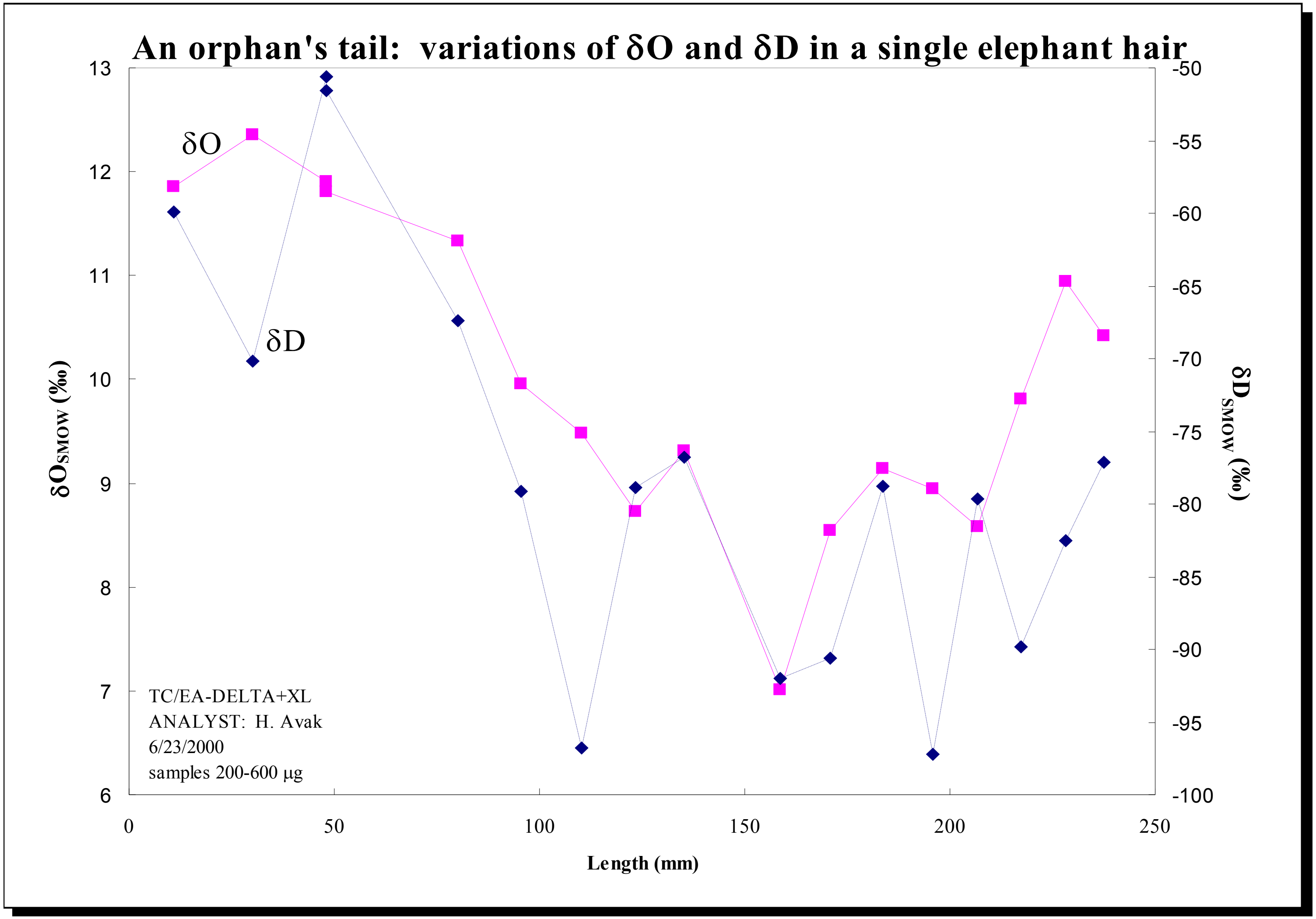


^{18}O/^{16}O для обычных и «органических» яиц



Возможности IRMS

TC/EA



Определение геграфического происхождения мяса птицы и говядины...

9. DETERMINING THE GEOGRAPHIC ORIGIN OF POULTRY MEAT AND DRIED BEEF BY A COMBINATION OF ELEMENT AND OXYGEN ISOTOPE ANALYSIS

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Key Words: poultry, dried beef, origin, elements, oxygen isotope

Introduction

European and Swiss legislation ask for traceability of food and because meat origin is an important criterion for consumers' purchase decision, the demand for tools to prove the geographic origin independently from mere paper traceability increases steadily. Also for PDO-labeled products a determination of the geographic origin of both meat origin and place of processing is desirable as it would protect regional producers and their products from potential frauds. First analyses gave indications that element and isotope analysis individually have quite a high potential to authenticate the geographic origin of meat (Franke et al., 2006; Franke et al., 2007). The aim of the present study was to test the extent to which combining such techniques might even improve the accuracy of this approach.

Material and Methods

A total of 78 poultry breasts were obtained from Brazil, France, Germany, Hungary, and Switzerland in two sample sets: the first comprising 22 samples from these countries (Phase I), the second 52 samples (Phase II). The authenticity of all samples had been certified with valid custom documents, specifying place and date of slaughter. Samples were vacuum-sealed and frozen at -25°C. Totally 71 dried beef meat samples (thereof 21 being collected in Phase I) were either directly collected from the production sites (samples produced in Switzerland) or purchased from producers in Australia, Austria, Canada, and USA between May 2004 and February 2006. The Austrian samples were produced from Brazilian raw meat, for the other non-Swiss samples raw meat originated from the country of processing. Swiss samples were partly produced in the Swiss canton of Valais using Swiss raw meat and partly in the canton of Grisons using both Swiss and Brazilian raw meat. Beef samples were vacuum-sealed and stored in a cooling room at 2.5 °C.

For element analysis between 0.4 - 0.5 g of dried beef and 0.8 - 1 g of poultry meat were subjected to micro-wave assisted pressure digestion with nitric acid and analyzed for a total of 48 elements/isotopes using a sector field Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; Element 2, Finnigan MAT, Bremen, D) (Franke et al., 2006). Between analyses of Phase I and II the method was adapted to the state of the art (software-update of ICP-MS, new microwave-oven). For oxygen isotope analysis the water was extracted out of approx. 10 g of the sample by azeotropic distillation (toluene, 18h, 130°C). The determination of the oxygen isotope ratio ($\delta^{18}O$) was carried out with Isotope Ratio Mass Spectrometry (IRMS; Delta-Plus XL, Finnigan, Bremen, D) (Franke et al., 2007). Each sample was analysed in duplicate. Multivariate statistical analysis were performed using Linear Discriminant Analysis (LDA) with stepwise backward elimination (probability to enter / to remove: 0.15) on the combined data set of element and isotope analyses. Afterwards a validation was carried out by determining the origin of the samples in Phase I based on the data of samples in Phase II.

Results and Discussion

For building the validation matrix LDA selected the elements ^{82}Se, ^{85}Rb, ^{88}Sr, ^{95}Mo, ^{142}Nd, ^{205}Tl, ^{23}Na, ^{44}Ca, ^{51}V, ^{75}As and $\delta^{18}O$. The results showed that it was possible to determine the origin of poultry breasts from the first sample set correctly at a mean rate of 55 % based on a statistical model build with samples of the second sample set (Table 1). Thereby samples from Brazil, France and Hungary could be classified at 100%, while samples from Germany were completely misclassified as being French. Swiss samples were misclassified as being Hungarian with the exception of one sample which was determined as being from Brazil. Reasons for the lack of discrimination between French and German samples might be the relatively close geographic proximity. However, it still was possible to separate Swiss samples from these origins despite comparable geographic distance. The reasons for the misclassification of the Swiss samples are not clear.

Table 1. Origin of poultry samples of Phase I as predicted based on results obtained in samples of Phase II.

	Brazil	France	Germany	Hungary	Switzerland	% correct
Brazil	4	0	0	0	0	100
France	0	2	0	0	0	100

Определение фальсификаций пищевых продуктов с использованием IRMS

Ann. Fac. Medic. Vet. di Parma (Vol. XXVI, 2006) pag. 193 - pag. 204

Stable isotopesdetermination in food authentication: a review

Ghidini S.°, Ianieri A.°, Zanardi E.°, Conter M.°, Boschetti T.*, Iacumin P.*, Bracchi. P. G.°

Introduction

Authenticity has probably always been a major concern of many consumers (Hargin, 1996), and it is still gaining more and more importance. In Europe, origin is one of the main authenticity issues dealing with food. The European legislation clearly shows this trend. As a matter of fact the trend can be detected already in horizontal sets of rules such as the white paper on food safety, and then in the commission Regulation 178/2002. Vertical sets of rules stress this tendency, for instance bovine meat Regulation CE 1760 17/07/2000 made the indication of origin on meat carcasses mandatory. Commission Directive 2001/110/CE posed the same condition for honey. The EU Commission regulation No 2065/2001 of 22 October 2001 has laid down detailed rules for the application of Council Regulation 104/2000 as regards informing consumers about fishery and aquaculture products. The information includes, between others, the area in which it was caught. In the case of cultivated species, the regulation indicates that a reference should be made to the country in which the product undergoes the final developmental stage.

High quality products with geographical indications and designations of origin following Commission Regulation 509/2006 are generally high-priced and bring in a higher benefit to the producers than ordinary products. So there is a need to protect such products by detecting possible commercial frauds. These products are defined by geographical origin, know-how and in some cases by feeding diet and animal breed.

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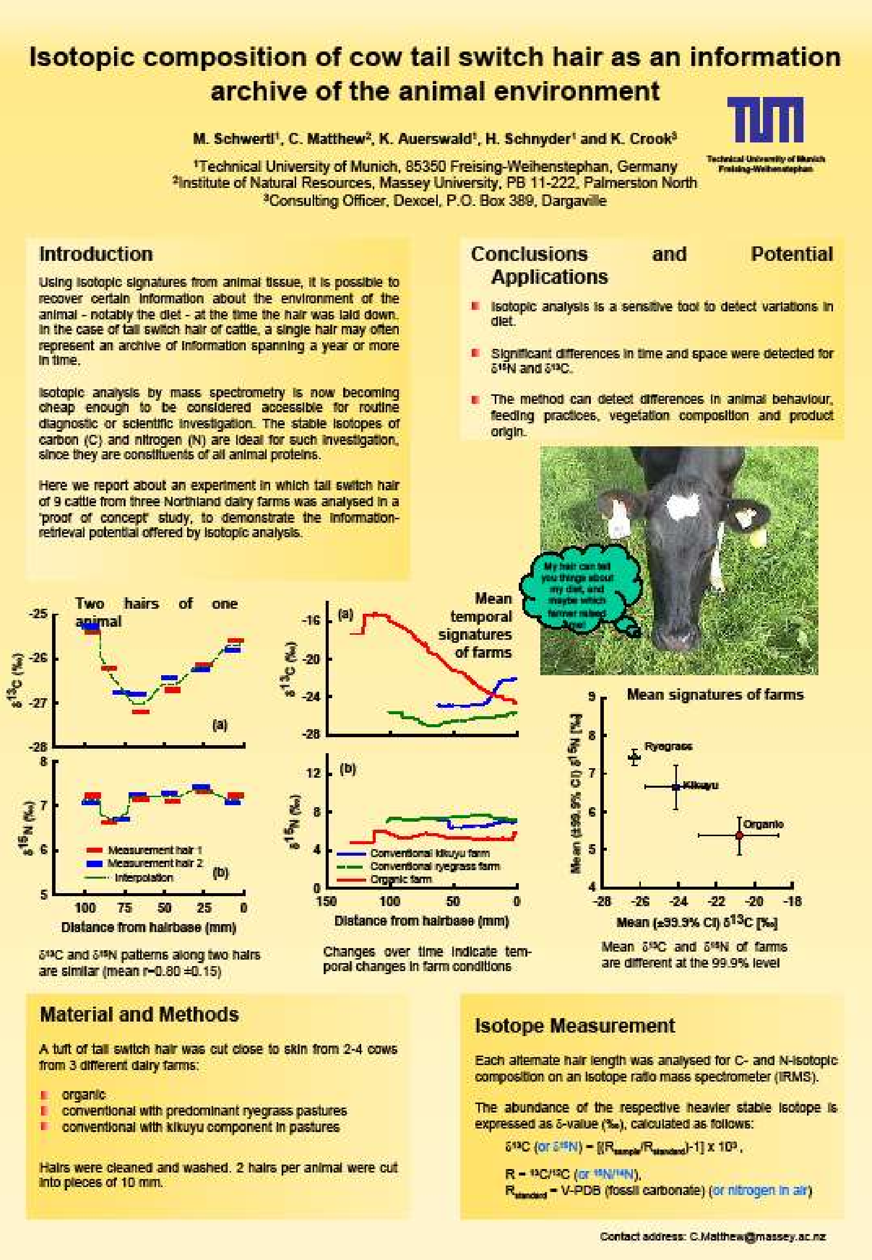
Изотопная композиция волоса из хвоста коровы как информационный архив условий выращивания



Isotopic composition of cow tail switch hair as an information archive of the animal environment

M. Schwertl[1], C. Matthew[2], K. Auerswald[1], H. Schnyder[1] and K. Crook[3]

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[2]Institute of Natural Resources, Massey University, PB 11-222, Palmerston North
[3]Consulting Officer, Dexcel, P.O. Box 389, Dargaville

Introduction

Using isotopic signatures from animal tissue, it is possible to recover certain information about the environment of the animal - notably the diet - at the time the hair was laid down. In the case of tail switch hair of cattle, a single hair may often represent an archive of information spanning a year or more in time.

Isotopic analysis by mass spectrometry is now becoming cheap enough to be considered accessible for routine diagnostic or scientific investigation. The stable isotopes of carbon (C) and nitrogen (N) are ideal for such investigation, since they are constituents of all animal proteins.

Here we report about an experiment in which tail switch hair of 9 cattle from three Northland dairy farms was analysed in a 'proof of concept' study, to demonstrate the information-retrieval potential offered by isotopic analysis.

Conclusions and Potential Applications

- Isotopic analysis is a sensitive tool to detect variations in diet.
- Significant differences in time and space were detected for $\delta^{15}N$ and $\delta^{13}C$.
- The method can detect differences in animal behaviour, feeding practices, vegetation composition and product origin.

$\delta^{13}C$ and $\delta^{15}N$ patterns along two hairs are similar (mean r=0.80 ±0.15)

Changes over time indicate temporal changes in farm conditions

Mean $\delta^{13}C$ and $\delta^{15}N$ of farms are different at the 99.9% level

Material and Methods

A tuft of tail switch hair was cut close to skin from 2-4 cows from 3 different dairy farms:

- organic
- conventional with predominant ryegrass pastures
- conventional with kikuyu component in pastures

Hairs were cleaned and washed. 2 hairs per animal were cut into pieces of 10 mm.

Isotope Measurement

Each alternate hair length was analysed for C- and N-isotopic composition on an isotope ratio mass spectrometer (IRMS).

The abundance of the respective heavier stable isotope is expressed as δ-value (‰), calculated as follows:

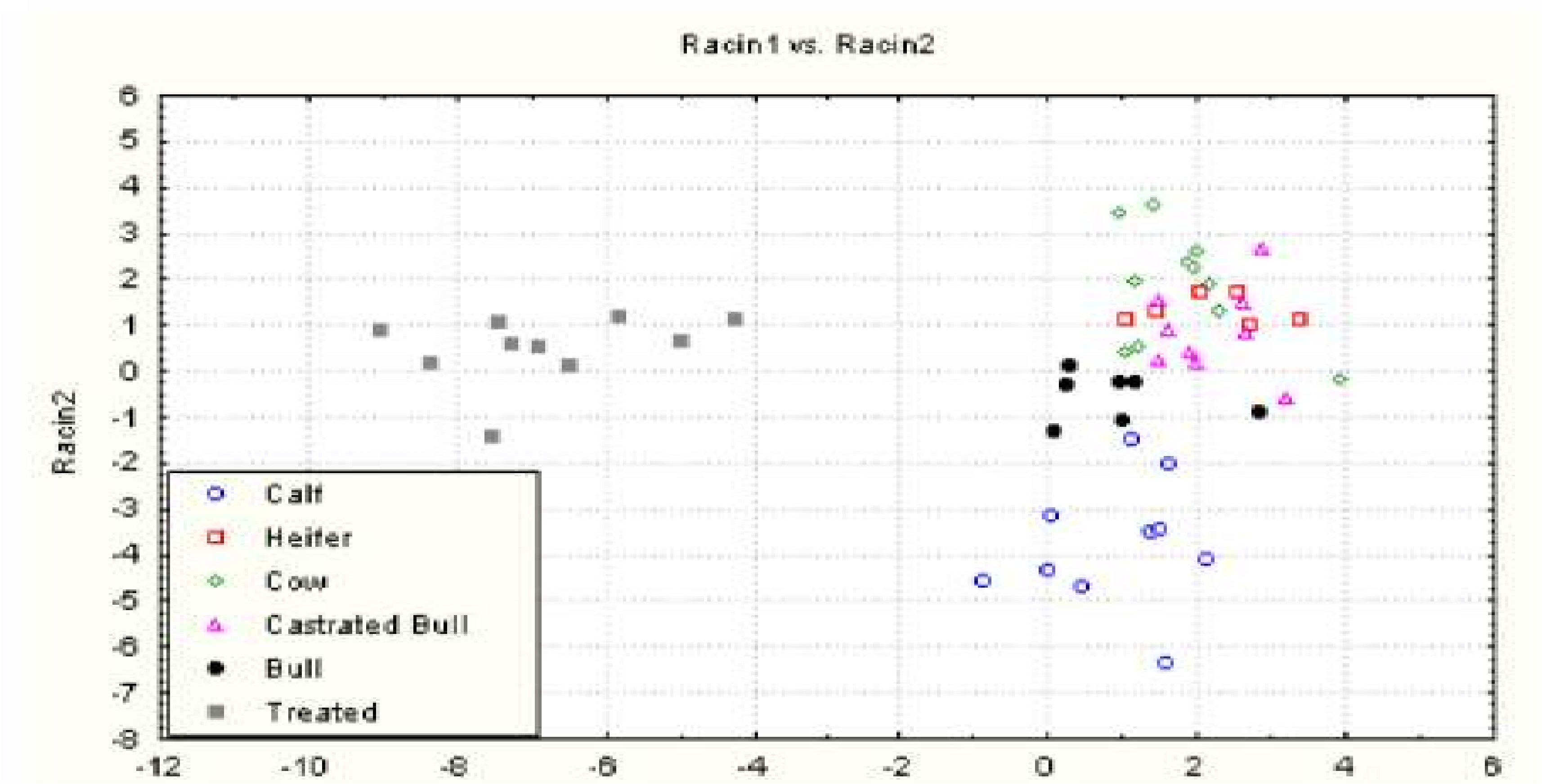
$\delta^{13}C$ (or $\delta^{15}N$) = $[(R_{sample}/R_{standard})-1] \times 10^3$,

R = $^{13}C/^{12}C$ (or $^{15}N/^{14}N$),
$R_{standard}$ = V-PDB (fossil carbonate) (or nitrogen in air)

Contact address: C.Matthew@massey.ac.nz

Сравнение изотопных отношений для мяса животных, которым не давали и давали стероидные гормоны

Final report ISOSTER Reporting Period 2002-2005



Образование тестостерона в организме

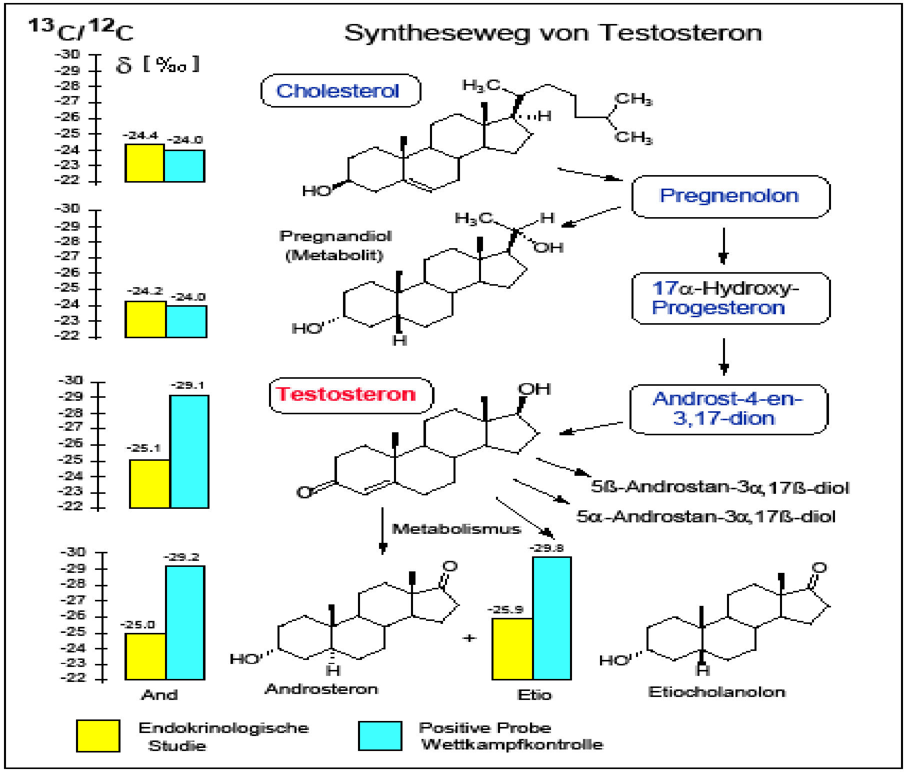


Abb.15

$^{13}C/^{12}C$-Isotopenverhältnisse endogener Steroidhormone eines Athleten, während einer endokrinologischen Studie und nach einem positiven Befund für Testosteron, im Vergleich zum Stoffwechselweg des Testosterons.

Заключение

Газовая масс-спектрометрия стабльных изотопов легких элементов является мощным и информационным инструментальным методом анализа и сочетается:

- с элементными анализаторами для интегрального изотопного анализа
- газовым и жидкостным хроматографами для изотпного анализа индивидуальных компонентов

Изотопная масс-спектрометрия обладает возможностями нести информацию о:

- **Географическом месте происхождения**
- **Фальсификации продукции**
- **Нелегальном использовании гормонов и других стероидных препаратов**

Спасибо за внимание!

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