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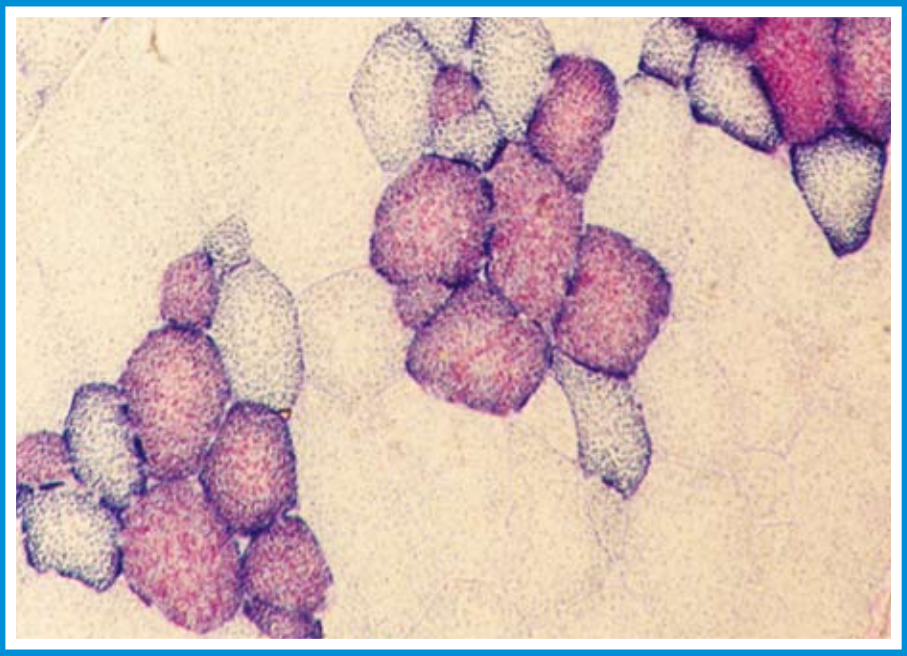
SLOVENSKI VETERINARSKI ZBORNIK



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SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

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Review Paper

RECORDS ON THE USE OF ANIMALS IN EXPERIMENTS IN THE REPUBLIC OF SLOVENIA AND IN OTHER EU MEMBER STATES WITHIN A 15-YEAR PERIOD

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Summary: Scope of this paper is to present data on the number and species of animals used in experiments and on the purposes of use of animals in the Republic of Slovenia and in the European Union within a 15-year period. According to data collected in the particular years of the period from 1992 to 2006 on the use of animals in experiments in the Republic of Slovenia, the trend of the use of animals in experiments has been found to be on the decline. The total number of experimental animals in 1992 amounted to 37,212, and in 2006 to 13,181 only. In the Republic of Slovenia, the use of experimental animals in applied research shows a downward trend on account of validated alternative methods in use, which do not require animals. In the most recent years in particular, the Slovenian legislative activity in the field of protection of experimental animals has been most productive with the scope of harmonising the Slovenian legislation with the EU law.
The European Commission published five reports on the use of animals in experiments. The total number of experimental animals as reported by the EU Member States amounted to 11.79 million in 1991, to 11.64 million in 1996, to 9.81 million in 1999, to 10.73 million in 2002, and to 12.11 million in 2005. The first two reports provided a limited scope of analysis due to the absence of a consistent system of reporting the data on the use of experimental animals in the Member States. The third and the fourth reports were based on agreed harmonized tables. This facilitated a more extensive interpretation of results on the use of experimental animals in the EU, despite some inconsistencies in the data submitted by the Member States. The second report covered for the first time the data collected by the 3 new Member States, and the fifth report by the 10 new Member States. However, it is not possible to draw conclusions on the evolution of use of animals for experimental purposes in the EU by comparing these data with those of the previous reports. The total number includes different animal species, from cold-blooded vertebrates on the one side, to mammals on the other, including farmed animals or anthropoid primates as used in some Member States. Comparison between the national reports is rendered impossible on account of the non-aligned methods of reporting by the EU Member States. These reports give a general survey only of the use of animals in experiments at Community level.

Key words: experimental animals; legislation; report

Background

In Europe and in other developed countries, methods are sought which would decrease the use of animals in experiments. Significant international development in regulating the protection of experimental animals was perceived at adoption of the European Convention (1) and Directive (2, 3). By Decision of the Council of Europe 1999/575/EC (4), the Community was signatory to the Convention of the Council of Europe, ETS 123 (1), increasing thereby its commitment to endeavours of replacing experimental animals and protecting those still used in experiments. The objective of Directive (2) and European Convention (1) was to provide for the harmonisation of provisions on the protection of experimental animals in the national legislations of the Member States. By complying with the proposed standards, the disparities would be abolished, and the measures for

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the protection of experimental animals harmonised in providing for the adequate conditions of rearing, care and use and, in particular, to avoid the unnecessary duplications of experiments on animals, by complying with the uniform standards and mutual recognition of test results obtained by experiments already conducted on animals. The requirement that experiments on animals shall not be conducted if another acceptable, feasible and scientifically satisfactory method is available, which does not require the use of experimental animals, has contributed to a decreased use of experimental animals and stimulated the development of alternative methods for experiments on animals. In 1991, the European Centre for the Validation of Alternative Methods – ECVAM was set up in Italy, which directly contributes to the protection of animals, using the 3 R concept (Replacement, Reduction, Refinement) and providing for the validation of alternative methods (5). Greater significance is awarded to the implementation and monitoring of policies in the different spheres of consumer protection and safety testing of chemicals, cosmetic products, biocides, foodstuffs, biological substances and medical devices. At implementation of relevant legislation on safety testing, the alternative methods, which exclude the use of experimental animals, are becoming more and more important. One of the seven main objectives of the action plan of the future policy on chemicals, set out in the White Paper of 2001 (6), is to support the developing of tests requiring no use of animals. Alternative methods can produce_reliable information in the most up-to-date and proven tests, which are more rapid and cost-effective than the existing experiments on animals. It has been estimated that the requirements (expenses and animals) for experiments within the REACH Programme (Registration, Evaluation and Authorisation of Chemicals) – including the registration, valuation, authorisation and restriction of chemicals, could decrease by 70 % if using the intelligent strategies for testing (7). A final goal is to replace_experiments on animals with methods that do not require the use of animals. At experiments which still do require the use of live animals, the goal is to decrease the number of animals used, and to improve the methods so as to cause less pain, suffering and harm. Activities towards the full implementation of the 3 R concept need to proceed in all the spheres of use of animals, the harmonisation of Directive 86/609/EEC (2) and legislation requiring experiments on animals shall be fully implemented, and agreements on the mutual acceptability and recognition of data shall be subjected to scrutiny. Setting up a Community Reference Laboratory for the validation of alternative methods shall additionally improve the quality of alternative methods for testing, and speed up the validation procedure (6, 7).

Legal basis for data collection on experimental animals

In Slovenia, the Veterinary Administration of the Republic of Slovenia keeps records based on annual reports by the user organisations, in compliance with Article 24 of the Animal Protection Act (8). In 2004, the methods of collecting data on experimental animals were laid down in the Rules (9, 10). Pending the entry into force of these Rules, the data were collected from the reports submitted by scientists performing a particular experiment. Using a questionnaire prepared to this end and partly summing up the European Convention, Appendix B (1), and including the visits to user organisations, data were gathered for the period from 1992 to 1996, and presented to the public for the first time in 1999 (11, 12). Data collected on entry into force of the Animal Protection Act (8) were made available to the public and to all the applicants as public information (13).

Based on Article 13 of Council Directive 86/609/EEC (2), the competent authorities of the EU Member States collect and make available to the public the statistical data on the use of animals in experiments. In Article 26, the said Directive is laying down that the European Commission shall prepare regular reports for the Council and the European Parliament, using the data presented by the EU Member States. As the Directive does not lay down the form and scope of reporting, a common form was unanimously adopted to this end after the multi-annual discussions of the national competent authorities. The European Union requires the reporting to be performed using eight tables, called the EU Tables. The European Convention (1) requires the member states of the Council of Europe to provide reports in five tables, called the Convention Tables. The reservation of the European Community as to the reporting remained unchanged in Council Decision 2003/584/EC (14) that is laying down the simplified procedure of amending the Appendices to the European Convention. The Republic of Slovenia, having ratified the European Convention in 2006 (15), provided as well for a reservation in accordance with Article 34 to the effect that it did not consider itself bound by the requirement of reporting statistical data.

Data on experimental animals used in the Republic of Slovenia in a 15-year period

Table 1 shows data on experimental animals used in the Republic of Slovenia in a 15-year period, collected by the Veterinary Administration of the Republic of Slovenia. In 1992, the number of experimental animals totalled 37,212, as compared to 2006 with 13,181 animals only, which is by 65 % less. Most used were the laboratory mice, followed by laboratory rats and rabbits. Within the first five years of data collection on experimental animals there stands out a high number of poultry. The numbers include also the poultry used in nutritional experiments under the normal rearing conditions and without the invasive treatment of animals. Such use of poultry was excluded from the subsequent reports. Table 1 includes a column on the protected animal species that are protected in the Republic of Slovenia in accordance with the Decree (16, 17). Of the protected animal species, mostly the amphibians (frogs) were used in experiments.

Most experiments are conducted on laboratory rodents in pharmaceutical industry for substance testing, and are carried out in accordance with the applicable legislation, the rules of pharmacopoeias applied, and international laws, regulations and administrative provisions. This information is evident from Table 2. Institutes and laboratories of university faculties of the medical, veterinary, biological and zootechnical programmes use animals in the baseline biological research, and in scientific and research studies. To a lesser extent, animals are used for diagnosing diseases, training and education, and for other purposes that are not particularly specified.

Table 1: Number and species of animals used in experiments in the Republic of Slovenia in the period from 1992 to 2006

Species	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Mice (Mus musculus)	19,754	16,475	15,555	16,274	15,163	15,233	11,796	12,900	10,394	9,773	9,024	8,388	7,560	8,556	7,590
Rats (Rattus norvegicus)	7,458	6,472	5,974	5,659	5,066	4,011	4,387	3,261	4,284	3,504	3,201	3,056	4,429	2,732	4,767
Guinea Pigs (Cavia porcellus)	532	553	450	567	482	400	468	139	79	50	112	120	121	38	26
Hamsters (Mesocricetus)	0	0	0	0	0	10	3	0	0	0	0	0	0	0	0
Other Rodents (other Rodentia)		6	86	0	0	0			10	0	0	177	35	18	0
Rabbits (Oryctolagus cuniculus)	1,251	1,207	1,387	909	1,451	1,107	1,439	781	744	712	795	597	582	533	472
Cats (Felis catus)	43	50	50	57	29	60	50	83	55	44	38	0	1	0	0
Dogs (Canis familiaris)	19	52	44	18	19	14	17	21	3	12	14	34	7	15	6
Horses, donkeys, crossbreeds (Equidae)					10	0	1	0	0	1	1	4	26	1	0
Pigs (Sus)	22	22	40	26	27	69	239	82	246	29	106	6	11	16	0
Goats (Capra)						0	0	0	10	60	0	0	0	0	0
Sheep (Ovis)	19	21	21	19	17	35	22	21	36	47	47	43	21	57	50
Cattle (Bos)	22	22	22	22	22	22	2	1	36	20	0	0	0	0	0
Primates (Prosimia)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poultry (Poultry)	8,004	7,394	8,524	8,575	7,625	361	520	334	60	438	225	0	0	0	0
Other birds (pigeon)						0	11	3,000	150	150	0	0	270	22	265
Fish (Pisces)	20	10	100	522	180	245	146	25	270	813	120	137	326	0	0
Other animals						706	606	756	172	287	236	81	4	0	4
Protected animals*	68	53	65	59	45	305	268	227	270	146	26	39	145	3	1
Total	**37,212**	**32,337**	**32,318**	**32,707**	**30,136**	**22,578**	**19,975**	**21,631**	**16,819**	**16,086**	**13,945**	**12,682**	**13,538**	**11,991**	**13,181**

Legend to Table 1: * Protected or endangered animal species in accordance with the Decree (16, 17) applicable at the time

Table 2: Number of animals used in experiments as to the purpose of use in the Republic of Slovenia in the period from 1992 to 2006

Research type	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Baseline biological research (1)	13,107	12,711	14,526	13,863	13,656	2,668	4,276	4,001	2,688	3,688	3,975	3,700	4,391	1,888	1,457
Applied research (2)	23,282	18,701	16,649	16,018	15,545	17,974	14,048	13,018	12,071	10,081	8,685	8,202	8,182	9,420	10,564
Research of substances for protecting the public, animals and environment (3)	130	50	140	539	200	477	305	273	859	1,467	120	0	0	*	*
Education and practice (4)	177	85	87	182	112	832	812	745	475	468	336	239	454	283	214
Forensics (5)	4	11	2	1	0	0	0	0	0	0	0	0	0	0	0
Diagnosing diseases (6)	512	779	914	2,104	623	539	331	565	368	267	339	446	414	378	683
Other (7)						88	203	3,029	358	115	490	95	97	22	263
Total	37,212	32,337	32,318	32,707	30,136	22,578	19,975	21,631	16,819	16,086	13,945	12,682	13,538	11,991	13,181

(1) Baseline biological research: research of the anatomy or operation of living organisms, organs, tissues and cells
(2) Applied research: research, development, quality control, pharmacological and toxicological analysing and testing of efficiency of medicinal products and medicinal substances in human medicine and dentistry, and in veterinary medicine.
(3) Research of substances for protecting the public, animals and environment with toxicological and other testing (excluding medicinal products and medicinal substances)
(4) Education and practice
(5) Forensics: human or veterinary
(6) Diagnosing diseases: tests for the specific determination of pathogens or for the production of diagnostic reagents
(7) Other: other purposes of use of animals in experiments.

* Number of animals used in the research of substances for protecting the public, animals and environment was in the period 2005 – 2006 included in the number of animals used in the applied research.

Data on experimental animals used in the European Union in a 15-year period

European Commission published five reports, which include data on experimental animals in the EU Member States. Table 3 shows the total number of animals used in experiments in the European Union in 1991, 1996, 1999, 2002 and 2005 (18, 19, 20, 21, 22).

The first report on animals used in experiments in the EU Member States (18) includes data on the total number of animals in 1991 only, and was published in 1994. France and Portugal reported the data of 1992, and Belgium and Luxemburg did not present any relevant reports. Total number of experimental animals used in 1991, as reported by the EU Member States, amounted to 11.79 million. Data are shown in column 2 of Table 3.

The second report on animals used in experiments in the EU Member States (19) provides data of 1996, and was published in 1999. The report includes the data of three new Member States, Austria, Finland and Sweden. France and Sweden presented their respective reports in the EU Tables, and the other Member States in the Convention Tables. France reported the data of 1997. This report and the subsequent reports include animals used for the education and training purposes. Such a purpose of use of animals is not laid down in Directive (2), but in Council Resolution 86/C331/02 (23). Some Member States included in their reports the animals which are neither indicated in the Directive nor in the Resolution, as for instance, the production of harmful mutants and transgenic animals. The number of animals used in experiments in the European Union in 1996 totalled 11.64 million. Table 4 shows relevant data.

The second report includes also the data on the purpose of use of animals in experiments, as shown in Table 5. Thirteen Member States reported on the purposes of use of animals in experiments. In case of some Member States, the data on the total number of animals used in experiments did not tally with the data on the purpose of use of animals in experiments. Per purpose of use of animals in experiments, the number of animals used in experiments in 1996 in the thirteen Member States totalled 8.81 million.

The third report on animals used in experiments in the EU Member States (20) provides the data of 1999, and was published in 2003. Member States presented their respective reports in the EU Tables, excluding one Member State that presented its re-

Table 3: Total number of animals used for experimental purposes in the EU Member States in 1991, 1996, 1999, 2002 and 2005

Abbreviation	Member State	1991	1996	1999	2002	2005
AT	Austria		204,825	130,295	192,062	167,312
BE	Belgium		1,515,867	790,089	695,091	718,976
CY	Cyprus					967
C Z	Czech Republic					330,933
DE	Germany	2,402,710	1,509,619	1,591,394	2,071,568	1,822,424
DK	Denmark	304,370	350,226	323,444	371,072	365,940
EL	Greece	25,439	19,280	9,686	515,423	926,092
ES	Spain	558,823	506,837	475,726	262,042	595,597
EE	Estonia					4,900
FR	France	3,645,708*	2,609,322**	2,309,597	2,212,294***	2,325,398
HU	Hungary					297,290
IE	Ireland	25,199	77,107	73,929	52,203	37,940
IT	Italy	683,293	1,094,185	987,771	924,889	896,966
LV	Latvia					13,319
LT	Lithuania					5,767
LU	Luxembourg		1,003	3,060	5,320	4,120
MT	Malta					0
NL	The Netherlands	876,058	652,300	621,466	640,930	531,199
PL	Poland					358,829
PT	Portugal	87,117*	49,520	39,851	44,577	41,621
FI	Finland		110,659	228,334	644,880	256,826
SI	Slovenia					11,991
SK	Slovakia					23,369
SE	Sweden		286,012	324,067	281,184	505,681
UK	United Kingdom	3,181,768	2,659,368	1,905,462	1,817,485	1,874,207
Total		**11,790,485**	**11,646,130**	**9,814,171**	**10,731,020**	**12,117,583**

* data of 1992
** data of 1997
*** data of 2001

port in the Convention Tables. The number of animals used in experiments in the European Union in 1999 totalled 9.81 million. Table 6 shows relevant data. The third report made by 14 Member States included also the data on the purpose of use of animals in experiments, as shown in Table 7. Data on the total number of animals used in experiments do not tally with the number of animals per purpose of use of animals in experiments.

The fourth report on animals used in experiments in the EU Member States (21), as shown in Table 8, provides the data of 2002, and was published in 2005. All the fifteen Member States presented their respective reports in the EU Tables, with the exception of France that reported the data of 2001. The number of animals used in experiments in the European Union in 2002 totalled 10.73 million. Part 2 of the report conveys the data of the particular Member States, including clarifications. Purposes of use of animals are described in detail, including the required conditions and types of testing.

The fourth report includes the data on the purpose of use of animals in experiments, as shown in Table 9. All the Member States reported on the purposes of use of animals in experiments. Data on the total number of animals used in experiments do tally with the data on the purposes of use.

Table 4: Total number of animals used for experimental purposes in 1996 in the EU Member States in 1996

Species	AT	BE	DE	DK	EL	ES	FR*	IE	IT	LU	NL	PT	FI	SE	UK	Total
Rodents and rabbits	200,640	711,748	1,258,110	307,513	17,091	481,950	2,411,358	54,925	1,071,856	1,003	500,720	46,567	76,759	266,922	2,348,758	**9,755,920**
Cold - blooded vertebrates (1)	2,158	736,165	134,952	24,604	1,930	1,090	103,024	19,021	9,193	0	44,787	118	29,608	11,489	146,924	**1,265,063**
Birds (2)	0	54,982	94,793	9,347	129	17,736	67,652	94	9,218	0	86,071	329	1,912	3,178	113,691	**459,132**
Artio + Perissodactyla (3)	0	9,073	14,026	7,028	126	5,126	18,054	2,554	1,868	0	17,865	2,457	2,097	3,070	32,413	**115,757**
Carnivores (4)	274	2,899	5,887	1,710	2	812	6,545	513	1,254	0	1,763	44	248	1,266	12,980	**36,197**
Prosimians + Monkeys + apes	164	600	1,519	18	2	53	2,622	0	772	0	1,082	0	17	46	3,786	**10,681**
Other animals	1,589	400	332	6	0	70	67	0	24	0	12	5	18	41	816	**3,380**
Total	**204,825**	**1,515,867**	**1,509,619**	**350,226**	**19,280**	**506,837**	**2,609,322**	**77,107**	**1,094,185**	**1,003**	**652,300**	**49,520**	**110,659**	**286,012**	**2,659,368**	11,646,130

Species %	AT	BE	DE	DK	EL	ES	FR*	IE	IT	LU	NL	PT	FI	SE	UK	Mean
Rodents and rabbits	97.96	46.95	83.34	87.80	88.65	95.09	92.41	71.23	97.96	100	76.76	94.04	69.37	93.33	88.32	**83.77**
Cold - blooded vertebrates (1)	1.05	48.56	8.94	7.03	10.01	0.22	3.95	24.67	0.84	0.00	6.87	0.24	26.76	4.02	5.52	**10.86**
Birds (2)	0.00	3.63	6.28	2.67	0.67	3.50	2.59	0.12	0.84	0.00	13.20	0.66	1.73	1.11	4.28	**3.94**
Artio + Perissodactyla (3)	0.00	0.60	0.93	2.01	0.65	1.01	0.69	3.31	0.17	0.00	2.74	4.96	1.90	1.07	1.22	**0.99**
Carnivores (4)	0.13	0.19	0.39	0.49	0.01	0.16	0.25	0.67	0.11	0.00	0.27	0.09	0.22	0.44	0.49	**0.31**
Prosimians + Monkeys + apes	0.08	0.04	0.10	0.01	0.01	0.01	0.10	0.00	0.07	0.00	0.17	0.00	0.02	0.02	0.14	**0.09**
Other animals	0.78	0.03	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.03	**0.03**
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**100**

* = France reporting for 1997
For abbreviations see Table 3
(1) = reptiles + amphibians + fish
(2) = quails and other birds
(3) = horses, donkeys and crossbreeds + pigs + goats and sheep + cattle
(4) = cats + dogs + ferrets + other carnivores

Table 5: Number of animals used for selected purposes versus species in the EU Member States in 1996

Species	Baseline biological research	Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine	Toxicological and other safety valuations (including valuation of products)	Diagnosis of disease	Education and training	Other	Total
Rodents and rabbits	1,820,483	3,644,125	536,527	317,145	75,090	751,776	**7,145,146**
Cold - blooded vertebrates (1)	190,605	23,458	59,750	8,257	5,699	661,885	**949,654**
Birds (2)	64,592	68,256	6,226	5,406	936	45,423	**190,839**
Artio + Perissodactyla (3)	22,963	17,753	739	5,427	2,476	8,855	**58,213**
Carnivores (4)	8,381	14,982	3,403	814	693	211	**28,484**
Prosimians + Monkeys + apes	1,187	5,737	631	254	41	242	**8,092**
Other animals	98,561	129,136	152,167	51,064	7,019	1,337	**439,284**
Total	**2,206,772**	**3,903,447**	**759,443**	**388,367**	**91,954**	**1,469,729**	8,819,712

Table 6: Total number of animals used for experimental purposes in 1999 in the EU Member States

Species	AT	BE	DE	DK	EL	ES	FR	IE	IT	LU	NL	PT	FI	SE	UK	Total
Mice	91,194	446,677	775,932	163,680	3,566	261,301	1,552,330	31,251	410,788	3,000	277,774	23,669	89,959	184,230	990,162	**5,305,513**
Rats	12,699	169,662	403,227	96,864	1,900	134,070	460,407	14,484	500,625	20	159,768	9,836	32,519	84,374	526,904	**2,607,349**
Guinea - Pigs	7,367	37,397	42,891	10,431	240	13,892	77,021	1,041	18,474	20	10,246	1,452	1,737	9,355	61,308	**292,872**
Other Rodents	396	19,641	18,020	1,310	0	1,227	25,605	133	6,023	0	5,267	1,211	1,763	550	18,848	**99,994**
Rabbits	15,056	20,968	50,623	6,543	632	19,496	49,836	915	19,030	20	9,222	730	1,686	5,031	27,578	**227,366**
Cold - blooded vertebrates (1)	1,447	65,097	179,869	29,018	1,840	20,605	29,042	20,052	7,995	0	47,428	539	89,094	28,249	130,595	**650,870**
Birds (2)	1,367	19,726	92,792	5,225	80	19,027	86,610	1,229	20,157	0	92,823	267	5,228	6,920	105,931	**457,382**
Artio+ Perisso-dactyla (3)	670	8,874	17,765	9,004	1,426	4,181	18,735	4,370	3,295	0	17,430	1,752	2,347	4,165	29,376	**123,390**
Carnivores (4)	92	1,557	7,531	1,358	2	1,831	7,417	441	847	0	1,153	94	1,844	774	10,632	**35,573**
Prosimians + Monkeys+apes	7	490	2,084	0	0	96	2,322	0	512	0	320	0	9	66	3,191	**9,097**
Other Mammals	0	0	660	11		0	272	13	25	0	45	301	2,148	353	937	**4,765**
Total	**130,295**	**790,089**	**1,591,394**	**323,444**	**9,686**	**475,726**	**2,309,597**	**73,929**	**987,771**	**3,060**	**621,466**	**39,851**	**228,334**	**324,067**	**1,905,462**	**9,814,171**

Species %	AT	BE	DE	DK	EL	ES	FR	IE	IT	LU	NL	PT	FI	SE	UK	Mean
Mice	69.99	56.54	48.76	50.61	36.82	54.93	67.21	42.27	41.59	98.04	44.70	59.39	39.40	56.85	51.96	**54.06**
Rats	9.75	21.47	25.34	29.95	19.62	28.18	19.93	19.59	50.68	0.65	25.71	24.68	14.24	26.04	27.65	**26.57**
Guinea - pigs	5.65	4.73	2.70	3.22	2.48	2.92	3.33	1.41	1.87	0.65	1.65	3.64	0.76	2.89	3.22	**2.98**
Other rodents	0.30	2.49	1.13	0.41	0.00	0.26	1.11	0.18	0.61	0.00	0.85	3.04	0.77	0.17	0.99	**1.02**
Rabbits	11.56	2.65	3.18	2.02	6.52	4.10	2.16	1.24	1.93	0.65	1.48	1.83	0.74	1.55	1.45	**2.32**
Cold - blooded vertebrates (1)	1.11	8.24	11.30	8.97	19.00	4.33	1.26	27.12	0.81	0.00	7.63	1.35	39.02	8.72	6.85	**6.63**
Birds (2)	1.05	2.50	5.83	1.62	0.83	4.00	3.75	1.66	2.04	0.00	14.94	0.67	2.29	2.14	5.56	**4.66**
Artio +Perisso-dactyla (3)	0.51	1.12	1.12	2.78	14.72	0.88	0.81	5.91	0.33	0.00	2.80	4.40	1.03	1.29	1.54	**1.26**
Carnivores (4)	0.07	0.20	0.47	0.42	0.02	0.38	0.32	0.60	0.09	0.00	0.19	0.24	0.81	0.24	0.56	**0.36**
Prosimians + Monkeys+apes	0.01	0.06	0.13	0.00	0.00	0.02	0.10	0.00	0.05	0.00	0.05	0.00	0.00	0.02	0.17	**0.09**
Other Mammals	0.00	0.00	0.04	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.76	0.94	0.11	0.05	**0.05**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

For abbreviations see Table 3

Table 7: Number of animals used for selected purposes versus species in the EU Member States in 1999

Species	Baseline biological research	Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine	Toxicological and other safety valuations (including safety valuation of products)	Diagnosis of disease	Education and training	Other	Total
Mice	1,452,583	2,347,842	285,132	93,218	27,719	219,937	**4,426,431**
Rats	567,904	1,265,125	284,940	4,837	36,157	24,959	**2,183,922**
Other Rodents	40,631	215,796	51,397	3,618	1,571	11,897	**324,910**
Rabbits	22,701	84,159	30,104	9,108	3,316	9,850	**159,238**
Cold - blooded vertebrates (1)	215,412	56,186	82,113	21,317	11,300	82,470	**468,798**
Birds (2)	101,487	165,879	18,571	4,107	1,707	71,472	**363,223**
Artio + Perissodactyla (3)	45,687	34,135	3,584	3,573	4,824	13,129	**104,932**
Carnivores (4)	6,930	8,963	9,190	221	594	1,995	**27,893**
Prosimians + Monkeys + apes	1,279	1,796	3,687	22	4	206	**6,994**
Other Mammals	3,430	312	274	0	0	89	**4,105**
Total	**2,458,044**	**4,180,193**	**768,992**	**140,021**	**87,192**	**436,004**	**8,070,446**

Table 8: Total number of animals used for experimental purposes in the EU Member States in 2002

Species	AT	BE	DE	DK	EL	ES	FR*	IE	IT	LU	NL	PT	FI	SE	UK	Total
Mice	153,034	460,487	1,071,282	221,557	3,589	200,821	1,370,293	16,790	466,640	3,000	288,706	27,616	98,078	163,041	914,795	**5,459,729**
Rats	13,175	116,340	483,470	80,518	4,021	38,544	471,234	8,282	377,573	2,200	128,975	12,302	27,563	73,862	473,285	**2,311,344**
Guinea - Pigs	7,566	34,305	39,913	7,613	310	1,932	59,184	35	18,722	100	8,752	633	757	2,738	43,779	**226,339**
Other Rodents	132	19,315	24,057	6,966	135	587	24,099	6	9,106	0	7,788	93	3,822	1,283	13,820	**111,209**
Rabbits	15,560	10,805	132,833	5,542	1,492	2,292	53,545	130	12,481	20	8,093	908	1,235	2,165	20,574	**267,675**
Cold - blooded vertebrates (1)	1,176	26,235	208,805	36,171	502,360	14,888	109,831	21,046	6,202	0	32,426	2,399	502,400	19,383	165,938	**1,649,260**
Birds (2)	417	20,352	78,882	5,275	340	1,625	94,932	0	28,892	0	143,100	198	6,872	14,053	140,029	**534,967**
Artio + Perisso dactyla (3)	536	5,486	22,867	6,621	3,141	1,138	17,770	5,520	3,771	0	20,761	394	2,969	3,422	32,009	**126,405**
Carnivores (4)	388	1,191	6,468	794	35	141	7,518	262	1,071	0	1,968	34	494	1,049	8,699	**30,112**
Prosimians+ Monkeys+apes	78	567	1,844	5	0	74	3,840	0	420	0	270	0	0	91	3,173	**10,362**
Other Mammals	0	8	1,147	10			48	132	11		91		690	97	1,384	**3,618**
Total	192,062	695,091	2,071,568	371,072	515,423	262,042	2,212,294	52,203	924,889	5,320	640,930	44,577	644,880	281,184	1,817,485	**1,0731,020**

Species %	AT	BE	DE	DK	EL	ES	FR*	IE	IT	LU	NL	PT	FI	SE	UK	Mean
Mice	79.68	66.25	51.71	59.71	0.70	76.64	61.94	32.16	50.45	56.39	45.04	61.95	15.21	57.98	50.33	**50.88**
Rats	6.86	16.74	23.34	21.70	0.78	14.71	21.30	15.86	40.82	41.35	20.12	27.60	4.27	26.27	26.04	**21.54**
Guinea - Pigs	3.94	4.94	1.93	2.05	0.06	0.74	2.68	0.07	2.02	1.88	1.37	1.42	0.12	0.97	2.41	**2.11**
Other Rodents	0.07	2.78	1.16	1.88	0.03	0.22	1.09	0.01	0.98	0.00	1.22	0.21	0.59	0.46	0.76	**1.04**
Rabbits	8.10	1.55	6.41	1.49	0.29	0.87	2.42	0.25	1.35	0.38	1.26	2.04	0.19	0.77	1.13	**2.49**
Cold - blooded vertebrates (1)	0.61	3.77	10.08	9.75	97.47	5.68	4.96	40.32	0.67	0.00	5.06	5.38	77.91	6.89	9.13	**15.37**
Birds (2)	0.22	2.93	3.81	1.42	0.07	0.62	4.29	0.00	3.12	0.00	22.33	0.44	1.07	5.00	7.70	**4.99**
Artio + Perisso dactyla (3)	0.28	0.79	1.10	1.78	0.61	0.43	0.80	10.57	0.41	0.00	3.24	0.88	0.46	1.22	1.76	**1.18**
Carnivores (4)	0.20	0.17	0.31	0.21	0.01	0.05	0.34	0.50	0.12	0.00	0.31	0.08	0.08	0.37	0.48	**0.28**
Prosimians + Monkeys +apes	0.04	0.08	0.09	0.00	0.00	0.03	0.17	0.00	0.05	0.00	0.04	0.00	0.00	0.03	0.17	**0.10**
Other Mammals	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.01	0.00	0.11	0.03	0.08	**0.03**
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**100**

* = France reporting for 2001
For abbreviations see Table 3

Table 9: Number of animals used for selected purposes versus species in the EU Member States in 2002

Species	Baseline biological research	Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine	Toxicological and other safety valuations (including safety valuation of products)	Diagnosis of disease	Education and training	Other	Total
Mice	2,125,001	2,473,444	358,090	187,231	54,716	261,297	5,459,779
Rats	638,337	1,196,783	375,656	8,548	52,062	39,908	2,311,294
Other Rodents	47,140	201,372	69,792	2,931	2,435	13,878	337,548
Rabbits	19,621	178,776	45,067	8,232	2,095	13,884	267,675
Cold - blooded vertebrates (1)	690,261	472,495	175,220	2,486	218,566	90,232	1,649,260
Birds (2)	141,623	197,706	18,975	6,631	4,934	165,098	534,967
Artio + Perissodactyla (3)	56,065	37,871	3,227	10,528	6,741	11,973	126,405
Carnivores (4)	5,754	9,202	13,188	712	408	848	30,112
Prosimians + Monkeys + apes	1,738	1,580	6,832	34	7	171	10,362
Other Mammals	2,886	58	0	0	3	671	3,618
Total	**3,728,426**	**4,769,287**	**1,066,047**	**227,333**	**341,967**	**597,960**	**1,0731,020**

* = France reporting for 2004

The fifth report on animals used in experiments in the EU Member States (22) provides the data of 2005, and was published in 2007. All the twenty-five Member States presented their respective reports in the EU Tables. France presented its report of 2004. This report includes the data presented by the ten new EU Member States, i.e. Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia and Slovenia. The number of animals used in experiments in the European Union in 2005 totalled 12.11 million. Data by the old Member States are presented in Table 10, and data by the new Member States in Table 11. The number of animals used in the ten new Member States represents 8.6 % of the total number of animals used in the twenty-five Member States. Part 2 of the report conveys data presented by the particular Member States, including clarifications. Purposes of use of animals are described in detail, including the required conditions and types of testing. Data on the total number of animals used in experiments do tally with the data on the purposes of use. Table 12 shows relevant data.

Table 10: Total number of animals used for experimental purposes in the 15 EU Member States reporting up to 2005

Species	AT	BE	DE	DK	EL	ES	FR*	IE	IT	LU	NL	PT	FI	SE	UK	Total
Mice	128,634	488,125	1,084,358	208,375	15,340	393,217	1,510,334	17,776	534,614	3,280	240,048	28,318	120,636	213,727	1,052,064	**6,038,846**
Rats	11,920	106,483	435,417	85,664	6,024	125,754	424,387	7,722	279,774	720	116,608	6,793	28,358	83,321	411,501	**2,130,446**
Guinea - Pigs	3,149	39,530	37,761	5,046	574	16,780	79,350	4	11,533	100	7,479	379	563	2,014	28,918	**233,180**
Other Rodents	224	4,134	15,538	6,783	40	1,202	21,374	0	3,840	0	8,411	129	3,313	1,436	11,962	**78,386**
Rabbits	18,439	21,159	103,329	5,805	1,255	11,878	93,282	379	9,916	20	8,251	594	1,214	2,112	15,523	**293,156**
Cold - blooded vertebrates (1)	2,104	40,286	74,905	36,852	902,275	31,013	66,072	6,420	19,598	0	18,076	4,799	93,240	188,545	203,173	**1,687,358**
Birds (2)	1,025	13,691	41,607	7,784	21	8,425	106,263	2,024	31,697	0	111,233	112	5,773	7,838	115,000	**452,493**
Artio + Perissodactyla (3)	1,664	3,530	20,622	8,603	548	6,094	13,540	3,281	4,420	0	18,963	460	2,569	4,378	22,787	**111,459**
Carnivores (4)	97	1,530	6,686	843	14	1,090	7,007	286	1,094	0	1,790	36	188	1,596	7,623	**29,880**
Prosimians+ Monkeys+apes	56	449	2,086	0	1	84	3,789	0	412	0	327	0	0	75	3,115	**10,394**
Other Mammals	0	59	115	185	0	60	0	48	68	0	13	1	972	639	2,541	**4,701**
Total	**167,312**	**718,976**	**1,822,424**	**365,940**	**926,092**	**595,597**	**2,325,398**	**37,940**	**896,966**	**4,120**	**531,199**	**41,621**	**256,826**	**505,681**	**1,874,207**	**1,1070,299**

Species %	AT	BE	DE	DK	EL	ES	FR	IE	IT	LU	NL	PT	FI	SE	UK	Mean
Mice	76.88	67.89	59.50	56.94	1.66	66.02	64.95	46.85	59.60	79.61	45.19	68.04	46.97	42.27	56.13	**53.07**
Rats	7.12	14.81	23.89	23.41	0.65	21.11	18.25	20.35	31.19	17.48	21.95	16.32	11.04	16.48	21.96	**19.28**
Guinea - Pigs	1.88	5.50	2.07	1.38	0.06	2.82	3.41	0.01	1.29	2.43	1.41	0.91	0.22	0.40	1.54	**2.12**
Other Rodents	0.13	0.57	0.85	1.85	0.00	0.20	0.92	0.00	0.43	0.00	1.58	0.31	1.29	0.28	0.64	**0.79**
Rabbits	11.02	2.94	5.67	1.59	0.14	1.99	4.01	1.00	1.11	0.49	1.55	1.43	0.47	0.42	0.83	**2.58**
Cold - blooded vertebrates (1)	1.26	5.60	4.11	10.07	97.43	5.21	2.84	16.92	2.18	0.00	3.40	11.53	36.30	37.29	10.84	**15.07**
Birds (2)	0.61	1.90	2.28	2.13	0.00	1.41	4.57	5.33	3.53	0.00	20.94	0.27	2.25	1.55	6.14	**5.44**
Artio + Perissodactyla (3)	0.99	0.49	1.13	2.35	0.06	1.02	0.58	8.65	0.49	0.00	3.57	1.11	1.00	0.87	1.22	**1.16**
Carnivores (4)	0.06	0.21	0.37	0.23	0.00	0.18	0.30	0.75	0.12	0.00	0.34	0.09	0.07	0.32	0.41	**0.33**
Prosimians + Monkeys + apes	0.03	0.06	0.11	0.00	0.00	0.01	0.16	0.00	0.05	0.00	0.06	0.00	0.00	0.01	0.17	**0.09**
Other Mammals	0.00	0.01	0.01	0.05	0.00	0.01	0.00	0.13	0.01	0.00	0.00	0.00	0.38	0.13	0.14	**0.08**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

* = France reporting for 2004
For abbreviations see Table 3

Table 11: Total number of animals used for experimental purposes in the 10 New Member States in 2005

Species %	**CY**	**CZ**	**EE**	**HU**	**LV**	**LT**	**MT**	**PL**	**SI**	**SK**	**Total**
Mice	967	82,252	4,350	138,312	10,480	5,116	0	126,492	8,556	14,975	**391,500**
Rats	0	31,703	484	109,479	2,376	493	0	51,558	2,732	6,761	**205,586**
Guinea - Pigs	0	4,075	0	8,360	297	0	0	10,763	38	594	**24,127**
Other Rodents	0	6,018	0	518	0	0	0	11,069	18	0	**17,623**
Rabbits	0	5,567	66	9,152	166	158	0	3,101	533	782	**19,525**
Cold - blooded vertebrates (1)	0	71,186	0	11,315	0	0	0	56,413	3	0	**138,917**
Birds (2)	0	126,241	0	17,434	0	0	0	62,618	22	251	**206,566**
Artio + Perissodactyla (3)	0	3,193	0	1,303	0	0	0	24,026	74	0	**28,596**
Carnivores (4)	0	459	0	1,330	0	0	0	7,728	15	6	**9,538**
Prosimians + Monkeys + apes	0	51	0	6	0	0	0	0	0	0	**57**
Other Mammals	0	188	0	0	0	0	0	5,061	0	0	**5,249**
Total	**967**	**330,933**	**4,900**	**297,209**	**13,319**	**5,767**	**0**	**358,829**	**11,991**	**23,369**	**1,047,284**

Species %	CY	CZ	EE	HU	LV	LT	MT	PL	SI	SK	**Mean**
Mice	100	24.85	88.78	46.54	78.68	88.71	0	35.25	71.35	64.08	**37.38**
Rats	0.00	9.58	9.88	36.84	17.84	8.55	0	14.37	22.78	28.93	**19.63**
Guinea - Pigs	0.00	1.23	0.00	2.81	2.23	0.00	0	3.00	0.32	2.54	**2.30**
Other Rodents	0.00	1.82	0.00	0.17	0.00	0.00	0	3.08	0.15	0.00	**1.68**
Rabbits	0.00	1.68	1.35	3.08	1.25	2.74	0	0.86	4.45	3.35	**1.86**
Cold - blooded vertebrates (1)	0.00	21.51	0.00	3.81	0.00	0.00	0	15.72	0.03	0.00	**13.26**
Birds (2)	0.00	38.15	0.00	5.87	0.00	0.00	0	17.45	0.18	1.07	**19.72**
Artio + Perissodactyla (3)	0.00	0.96	0.00	0.44	0.00	0.00	0	6.70	0.62	0.00	**2.73**
Carnivores (4)	0.00	0.14	0.00	0.45	0.00	0.00	0	2.15	0.13	0.03	**0.91**
Prosimians + Monkeys + apes	0.00	0.02	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	**0.01**
Other Mammals	0.00	0.06	0.00	0.00	0.00	0.00	0	1.41	0.00	0.00	**0.5**
Total	100	100	100	100	100	100	0	100	100	100	**100**

Table 12: Number of animals used for selected purposes versus species in the all EU Member States* in 2005

Species	Baseline biological research	Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine	Toxicological and other safety valuations (including safety valuation of products	Diagnosis of disease	Education and training	Other	Total
Mice (Mus musculus)	2,465,474	2,727,254	384,741	225,524	86,597	551,356	6,440,946
Rats (Rattus norvegicus)	677,533	1,161,517	350,275	13,564	50,048	72,876	2,325,813
Other Rodents (other Rodentia)	53,241	230,403	56,006	4,512	2,606	6,548	353,316
Rabbits (Oryctolagus cuniculus)	15,463	237,411	38,761	8,322	3,856	8,829	312,642
Cold - blooded vertebrates (1)	485,858	942,973	116,123	5,905	40,236	235,180	1,826,275
Birds (Aves) (2)	251,443	249,024	53,935	9,723	5,440	89,494	659,059
Artio + Perissodactyla (3)	64,419	41,079	4,542	4,100	9,491	16,341	139,972
Carnivores (Carnivore) (4)	11,605	9,309	14,884	348	674	2,339	39,159
Prosimians + Monkeys + apes	1,456	1,397	7,004	16	42	536	10,451
Other Mammals (other Mammalia)	8,978	214	15	0	4	739	9,950
Total	**4,035,470**	**5,600,581**	**1,026,286**	**272,014**	**198,994**	**984,238**	**12,117,583**

* = France reporting for 2004

Table 13 shows the comparison between proportions of groups of animals used in the EU in experiments in 1996, 1999, 2002 and 2005 (22). As seen above, the most used group of animals represent the rodents and rabbits with around 80 %, with the highest use in 1999 and the lowest use in 2005. The second most used group of animals represent cold-blooded vertebrates, and their use ranges between 10 and 15 %, with the rather low use in 1999. Birds represent a third most used group of animals, which ranges between 4.7 and 5.4 %. A fourth most used group represent the equidae and ungulates with around 1 %.

Table 13: Comparison between proportions of classes of animals used in EU in 1996, 1999, 2002 and 2005

Class of species	1996*	1999	2002**	2005***
% Rodents - rabbits (1)	81.3	86.9	78.0	77.5
% Cold - blooded vertebrates (2)	12.9	6.6	15.4	15
% Birds (3)		4.7	5	5.4
% Artio + Perissodactyla (4)		1.2	1.2	1.1

* = 14 Member States reporting for 1996; 1 for 1997
** = 4 Member States reporting for 2002; 1 for 2001
*** = 24 Member States reporting for 2005; 1 for 2004

Discussion

In the Republic of Slovenia, the data on animals used in experiments have been known since 1992; however, the data collection method was defined in 2004 only (in a specific regulation). Data collected in the Republic of Slovenia show a downward trend in animal use in experiments. In the beginning of data collection it was believed that the number of animals used in experiments would increase from year to year owing to the more comprehensive methods of data collection, though the real number of animals

used in experiments would be smaller. However, the presentation of the total number of animals used in experiments, by species and in a longer period of time, clearly demonstrates the opposite. In the Republic of Slovenia, the use of experimental animals in applied research shows a downward trend on account of validated alternative methods in use, which do not require animals, even if authorised for use in experiments by the law. In the most recent years in particular, the Slovenian legislative activity in the field of protection of experimental animals has been most productive with the scope of harmonising the Slovenian legislation with the EU law. Current activities are focused on improving the minimum accommodation standards and conditions of care of particular animal species, including those not covered by the applicable legislation. This is resulting from the Protocol of Amendment (ETS 170) to Convention (ETS 123) of the Council of Europe (23), which was fully transposed as recommendation by the European Commission (24) in Directive 86/609/EEC (2).

A more extensive amendment of the applicable legislation is envisaged to take place, including the European Commission's proposal for a directive amending Directive 86/609/EEC. The proposed draft Directive (25) shall take into account the most recent developments in animal welfare and ethical concerns of animal use in experiments. The proposed draft Directive shall harmonise disparities between the national laws of the EU Member States so as to harmonise actions of protecting experimental animals, decreasing the number of animals used in experiments, and avoiding the unnecessary duplication of experiments. The Republic of Slovenia supports the strategy of protecting experimental animals, by urgently requiring the numbers of animals used in experiments to decrease, by introducing alternative methods and providing for the utmost protection and, at the same time, by providing for the welfare of animals which are still used in experiments on the reasonable and justifiable grounds.

Reviewing the number of animals used in experiments in the particular EU Member States through all the five years of reporting, it may be established that the number of animals used in experiments has been oscillating in many Member States. Most animals were used in experiments in France, United Kingdom and in Germany. Data presented by the Member States show a general survey only of the use of animals in experiments in their respective countries. Data cannot be compared on account of the differing reporting methods. For this very reason it is important that the EU Member States present the data in a standardised way so as to facilitate comparison. It may be envisaged with certainty that the number of animals used in experiments will decrease in future, in the Republic of Slovenia as well as in the European Union. This fact will be influenced by the more rigorous legislation, more severe inspection and control, replacement of animals by alternative methods, authorisation granting procedures for the implementation of experiments which will take into account the opinions stated by the ethical commissions, staff qualification, higher responsibility of the researchers and their improved attitude towards experimental animals that shows in preparing the precise experimental protocols, in selecting the methods and in the implementation of experiments as such. In decreasing the numbers of experimental animals, the mutual cooperation of institutions, researchers at state and interstate levels, as well as active approach of animal protection societies will be of key importance. In decreasing the numbers of experimental animals in pharmaceutical industry in particular, in addition to the validated alternative methods, the interstate recognition of results obtained by experiments already conducted on animals, the improved biometric methods, improved baseline research stages of new substances, and the use of cell cultures, tissues or smaller groups of animals play a significant role.

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POROČILA O UPORABI ŽIVALI V POSKUSIH V REPUBLIKI SLOVENIJI IN DRUGIH DRŽAVAH, ČLANICAH EVROPSKE UNIJE V 15-LETNEM OBDOBJU

D. Ornik, V. Čadonič-Špelič

Povzetek: Namen prispevka je prikazati podatke o številu in vrsti uporabljenih živali v poskusih ter o namenih uporabe živali v Republiki Sloveniji in Evropski uniji v obdobju 15 let. V Republiki Sloveniji na podlagi zbranih podatkov v letih od 1992 do 2006 ugotavljamo, da trend uporabe poskusnih živali pada. V letu 1992 jih je bilo 37.212, v letu 2006 pa le 13.181. V Sloveniji upada uporaba poskusnih živali zaradi uporabe validiranih alternativnih metod, ki ne zahtevajo živali. Zlasti v zadnjih nekaj letih poteka v Sloveniji aktivna zakonodajna dejavnost na področju zaščite poskusnih živali, da bi harmonizirali slovensko zakonodajo z evropsko.

Evropska komisija je objavila pet poročil o uporabi živali v poskusih. Kot so poročale države članice, je bilo v letu 1991 11,79 milijona poskusnih živali, v letu 1996 11,64 milijona, v letu 1999 9,81 milijona, v letu 2002 10,73 milijona in v letu 2005 12,11 milijona. Prvi dve poročili dajeta nepopolno analizo zaradi neenotnega navajanja podatkov o uporabi poskusnih živali v državah članicah. Tretje in četrto poročilo temeljita na dogovorjenih enotnih tabelah. To omogoča razširjeno obrazložitev zbranih podatkov o uporabi poskusnih živali v Evropski uniji, kljub določenim neskladnostim pri poročanju držav članic. Drugo poročilo prvič vsebuje podatke, zbrane v treh novih državah članicah, peto poročilo pa v desetih novih državah članicah. Podatkov o uporabi živali v poskusne namene ni mogoče primerjati s tistimi iz prejšnjih poročil. Skupno število vključuje različne živalske vrste, od hladnokrvnih vretenčarjev na eni strani do sesalcev, kot so rejne živali ali človeku podobni primati v nekaterih državah članicah na drugi strani. Zaradi neenotnega poročanja držav članic primerjava med nacionalnimi poročili ni mogoča. Poročila dajejo le splošen pregled nad uporabo poskusnih živali na nivoju skupnosti.

Ključne besede: poskusne živali; zakonodaja; poročilo

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Review Paper

ENZYME-IMMUNOHISTOCHEMICAL ASPECTS OF MUSCLE FIBER TYPE CLASSIFICATION IN MAMMALS

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Summary: Skeletal muscles are the most abundant and adaptable tissue in mammalians. They are composed of heterogeneous muscle fibers, in which distinct sets of structural proteins and metabolic enzymes are expressed. The percentages of different muscle fibers in the muscle define its morphological and functional characteristics. In this review, we summarize enzyme-immunohistochemical techniques to present muscle fiber type characteristics and their diversity in somatic skeletal muscles of various animal species. The principal methods to define myofiber properties on the tissue sections are based on the immunohistochemical determination of myosin heavy chain (MHC) isoforms and the myosin ATPase and metabolic enzyme histochemistry Four MHC isoforms (-I, -IIa, -IIx and -IIb) have been detected in somatic skeletal muscles of small mammals. Fibers that co-express more than one MHC isoform simultaneously are labeled as hybrid myofibers and are indicators of muscle fiber transition. The maximal shortening velocity of MHC fibers increases in the following order: -I < -IIa < -IIx < -IIb. On the basis of the myosin ATPase activity myofibers have been classified as types I, IIA, IIB and IIC. Type IIC fibers represent an intermediate type between MHC-I and type MHC-IIa fibers. Most large mammals do not possess fastest MHC-IIb isoform, although some recent studies in pigs and llamas have shown the existence of all three fast MHC isoforms in their skeletal muscles. Additional MHC isoforms are present transitorily during development, and in some highly functionally specialized muscles such as extraocular, laryngeal and masticatory muscles (MHC-extraocular, MHC-m). Embryonic and neonatal MHC isoforms are expressed during muscle development and regeneration. Slow MHC-I myofibers show high oxidative capacity, whereas fast MHC-II myofibers revealed entire spectrum of metabolic enzymes activity with large overlaps between contractile fiber types. Combining the contractile classification with metabolic enzymes activity, myofibers can be basically defined as slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast-twitch glycolytic (FG). In most cases enzyme and immunohistochemical techniques are not fully interchangeable, which makes combination of different techniques necessary to get a reliable classification of myofibers.

Key words: skeletal muscle; myosin heavy chain; muscle fiber type; histochemistry; mammals

Introduction

The principal muscle functional properties, such as contraction speed and fatigue resistance are mostly related to the proportions of myofiber types. Therefore, defining muscle fiber type composition became an essential step of any functional and applicative research in clinical and sports medicine as well as in animal muscle development and meat quality studies.

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In most mammals, skeletal muscle tissue represents about 55% of individual body mass and plays vital roles in locomotion, heat production and overall metabolism. In 19th century the French anatomist Louis Ranvier Antoine already observed that some muscles were darker and contracted more slowly during longer periods than paler muscles. This early observation was the basis for the distinction of red and white muscles, which was later found to be related to myoglobin content, an iron-containing oxygen transport protein in the muscle fibers (1). In the sixties and seventies of the last century, new histochemical procedures enabled to

distinguish muscle fibers on the basis of their contractile and metabolic properties. Furthermore, it was established that mammalian skeletal muscles were composed of different proportions of muscle fiber types, which define the properties of muscles as functional units. The proportions of the various myofiber types vary between muscles and between individuals for a given muscle (2). It is well known that endurance athletes have a greater proportion of slow-twitch oxidative fibers, whereas sprinters and weightlifters have more fast-twitch glycolytic fibers (3). Diverse myofiber type composition between individuals has been also reported in horses and dogs exhibiting different athletic abilities, as well as in different breeds of domestic pigs (4, 5).

On the cryosections the contractile properties of the myofibers are usually established either through immunohistochemical detection of the myosin heavy chain (MHC) isoforms or enzymehistochemical determination of myosin ATPase activity, while the energy metabolism is estimated on the basis of the histochemically demonstrated metabolic enzymes activity in the myofibers. Although all three techniques provide valuable data about the myofiber properties, the results could be sometimes erroneously interpreted, above all due to a lack of correspondence between myofiber classification systems within species and between species and because of antibodies immunoreactivity, which show certain diversity among various species (6).

Thus the main goal of this paper is to describe the cellular basis for the myofiber typing and present some particularities in the enzyme-immunohistochemical myofiber classification in different animal species.

Myosin heavy chain fiber type classification

The heterogeneity of mammalian skeletal muscle fibers is related to the diversity of myofibrillar proteins, predominantly the myosin heavy chain (MHC). Myosin is a large molecule composed of two myosin heavy chains: (200,000 kDa each) and four myosin light chains (MLC, app. 20 kDa) (7). MLC are divided into two alkali (essential) light chains and two regulatory light chains. The exact role of MLC in contraction is not fully established; however, it is assumed that they are involved in the regulation of shortening velocity of muscle fibers (8). The role of MHC is better established. It is both, a structural protein and an enzyme, which hydrolyses ATP and is therefore essential in determining excitation –contracting coupling and movement (9). It is well documented that MHC composition determines the force-velocity characteristics, making MHC composition a good tool to type myofibers functionally.

In mammalian skeletal muscles up to 9 MHC isoforms have been identified: -I, -IIa, -IIb, -IIx, -IIm, -neonatal, -embryonic, -extraocular and two cardiac. Each of them is encoded by a distinct gene and has its own myosin ATPase activity (10). They are grouped in clusters located in different chromosomes and forming distinct subfamilies. The subfamily of fast isoforms comprises genes coding for three fast isoforms (MHC-IIa, -IIx and -IIb) expressed in adult fast fibers of limb and trunk muscles and genes coding for extraocular, embryonic and neonatal isoforms. The subfamiliy od cardiac isoforms is composed of two genes, coding for slow (also β-cardiac) MHC expressed in cardiac muscle and in slow (type I) myofibers of skeletal muscles and for α-cardiac expressed in cardiomyocytes and in specialized skeletal muscles (masticatory, extraocular, laryngeal). Only the gene coding for masticatory (-IIm) MHC belongs to the third subfamily. This gene represents a autonomous subfamily because of the distinct chromosomal localization and also because sequence analysis carried out in cat, dog, and human shows a large diversity compared with all other MHC genes. (11).

The main MHC isoforms in adult locomotory skeletal muscle are -I, -IIa, -IIx and -IIb. MHC-I is a slow contracting isoform, while the three MHC-II isoforms are fast contracting; however, with different shortening speed. The polymorphism among adult MHC isoforms is functionally relevant as they determine not only myosin ATPase activity and fatigability, but the maximum shortening velocity of myofibers as well. Therefore, the existence of several MHC isoforms enables the skeletal muscles to fulfill different physiological demands. Studies on the isolated myfibers in rodents showed that the maximal shortening velocity increased in the following order: -I < -IIa < -IIx < -IIb (8, 12). Muscle fibers are capable of altering their phenotype under various conditions, such as altered neuromuscular activity, mechanical loading, hormonal profiles and aging. The changes in MHC isoforms follow a general scheme of reversible transitions from fast to slow and slow to fast in a order: MHC-I ↔ MHC-IIa ↔ MHC-IIx ↔ MHC-IIb (13, 14). The consequence of the MHC isoform tran-

sition scheme is that expression of two adjacent MHC isoforms in the same myofiber is possible. Such myofibers are designated as hybrid ones in contrast to so called pure myofibers, which contain only one MHC isoform. Recently, it has been established that different developmental and fast MHC isoforms could be co-expressed in the same muscle fiber during development, muscle regeneration and electrical stimulation, as well as in some highly specialized muscles such as extraocular, laryngeal and masticatory muscles (15).

Embryonic and neonatal MHC isoforms are typically expressed during muscle development and regeneration, yet they are also found in the intrafusal fibres (14). Masticatory (-IIm) MHC is phylogenetically ancient and confers high maximal muscle force and power. It is highly jaw-specific and is expressed in reptiles and fish. It is also found in several orders of mammals including carnivores, primates, chiropterans and diprotodonts.. In some species among listed mammals, masticatory myosin is replaced by some other isoform. It is postulated that during mammalian evolution, mastication of food became important, and in some yaw-closing muscles the masticatory myosin is replaced with α-cardiac, developmental, slow or fast limb isoforms to adapt to variety of diet (16, 17)

Extraocular MHC isoform has been shown in extraocular and some laryngeal muscles. The shortening speed of these muscles has proved to be even faster than that of masticatory ones (18, 19). Finally, α-cardiac MHC is fast contracting MHC isoform contained in cardiac muscle. It has been identified in adult human and rabbit masticatory muscles and is also temporally expressed during postnatal muscle development in pig and horse skeletal myofibers (20, 21) and in skeletal myofibers after chronic low frequency stimulation in rabbit (22). Currently, the shortening speed of adult MHC isoforms decrease in order -extraocular > -IIm > -IIb > -IIx > -IIa > α-cardiac > -I (23).

The expression of distinct MHC isoforms in myofibers, their function and the comparison of fiber type characteristics between different skeletal muscles and species has been studied using different procedures. Immunohistochemical detection with sets of monoclonal antibodies raised against different MHC is the most frequently used method to type myofibers. However, specifity of antibodies is still a problem in distinguishing between fast MHC isofoms in different species. Finally, *in situ* hybridization using nucleic acid probes for MHC isoforms and RT-PCR are used to analyze the expression of MHC at the mRNA level (24).

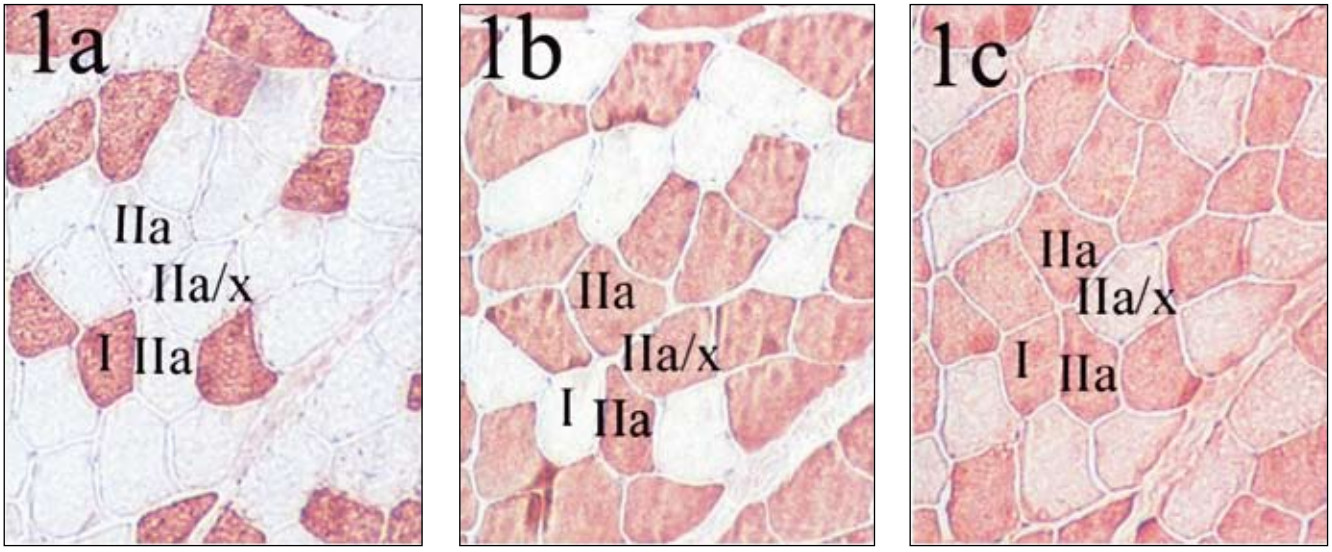

Figure 1: MHC isoforms expression in serial cross sections of canine triceps brachii muscle. MHC-I fibers were demonstrated using MHC-slow antibody (Fig. 1a). All myofibers that remained unstained were uniformly labeled with A4.74 antibody (fig 1b). The BF-35 antibody that recognizes all the MHC isoforms except -IIx, weakly stained a subpopulation of fast myofibers implying that they express the MHC-IIx isoform. Since these myofibers reacted positively with the A4.74 antibody, which is specific for MHC-IIa of rat, these fibers were first erroneously designated as hybrid co-expressing MHC-IIa and -IIx isoforms (MHC-IIa/x) (25). Further investigation showed that A4.74 antibody in canine muscles recognized both MHC-IIa and -IIx isoforms (26). Thus the BF-35 weakly stained myofibers were probably pure MHC-x myofibers, which were previously misclassified due to the nonspecific reaction of A,4.74 antibody

Myosin ATPase histochemistry

Barany (9) demonstrated that if myosin was extracted from skeletal muscle and activated in the presence of actin, the acto-myosin ATPase activity was directly proportional to the speed of shortening of the muscle from which the myosin was extracted. Since ATPase activity is ubiquitous in living organism, specific techniques to reveal myosin ATPase activity have been developed. They are all based on the precipitation of inorganic phosphate coming from the hydrolysis of ATP by myosin ATPase in the presence of Ca^{2+}. The staining procedure is performed on frozen unfixed sections, since fixation destroys enzyme activity. Subsequently, differences in the pH stability of myosin ATPase formed the basis for distinction of type I (slow) and type II (fast) myofibers (27). This method distinguished both fiber types at pH 9.4, because the fast type II fibers exhibited a much higher myosin ATPase activity at this pH than slow type I fibers. Further histochemical techniques based on propoperties of myosin ATPase activity revealed the presence of fast subtypes II fibers (28). Pre-incubation of serial cryosections in acid or alkali buffers before myosin ATPase staining could distinguish between type II fibers. Thus, three fast and one slow type could be demonstrated in small mammals (29, 30). The fast subtypes were shown to contain MHC-IIa, -IIx and -IIb, whereas the slow type contain MHC-I. These four types represent so called pure fibers containing only one MHC isoform. Alkali and acid stable type IIC fibers correspond to a hybrid myofiber population, containing both slow MHC-I and -IIa isoforms. Otherwise other hybrid fibers remain difficult to detect when using only myosin ATPase histochemisty. Their detection must be confirmed with complementary techniques such as immunohistochemistry. It should be stressed that interspecies differences exist for the pH stability/lability of myosin ATPase which makes identification of fiber types by myosin ATPase slightly different among species. (31).

Metabolic enzyme histochemistry

Another widely used method for determining the muscle fiber properties is histochemistry for selected enzymes of energy metabolism. Several metabolic enzymes have been chosen to represent metabolic pathways involve in either oxidative or glycolytic fuel utilization. Thus, different mitochondrial enzymes are markers of the potential oxidation of diverse substrates including fatty acids, carbohydrates and amino acids. Different enzymes of the glycolysis are used to determine the potential anaerobic catabolism of glycogen and glucose to lactate. In practice, succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (α-GPDH) are most frequently used to characterize oxidative and glycolytic potential capacities of myofibers, respectively (32, 33).

The combination of myosin ATPase with metabolic enzyme activities distinguishes, three basic muscle fiber types in mammalian muscles, i.e. slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast twich glycolytic (FG) (34). SO fibers are slow-contracting and are fatigue resistant. Structurally, they exhibit a small fiber diameter; possess a high mitochondrial and capillary density and a high myoglobin content. Energetically, these myofibers are rich in triglyceride droplets but have low level of glycogen and high energy creatine phosphate, which is usually used for explosive movements. Functionally, these fibers are used for aerobic activities like walking and maintaining posture. FOG fibers are fast contracting and resistant to fatigue. They have a high levels of mitochondria, capillary and myoglobine. They are rich in creatine phosphate and glycogen, moderately rich in triglycerides and exhibit an oxido-glycolytic metabolism. These myofibers are capable of prolonged anaerobic activity with a relatively high force output. FG fibers are fast contracting and very sensitive to fatigue. They have a low mitochondrial, capillary myoglobin and trigliceryde content (35) but exhibit high creatine phosphate and glycogen concentrations. FG fibers have large diameters and are used for short anaerobic activity with high force production such as galloping or jumping.

Slow type I myofibers are mostly oxidative and exhibit a rather uniform metabolic properties, whereas subtypes II fibers can be either oxido-glycolytic or glycolytic with large overlaps between subtypes. Moreover, MHC-IIb and -IIa fibers do not always correspond to FG and FOG fibers, respectively, and the discrepancy between myofiber classification becomes even more important when considering MHC-IIx myofibers. Therefore, the mixing of different classification systems can be misleading (36).

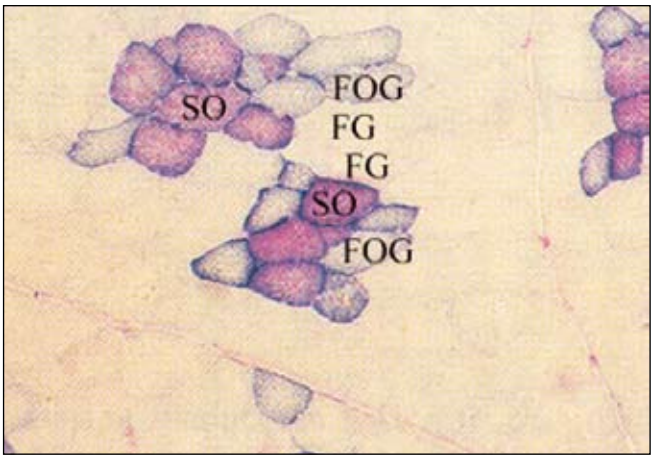

Figure 2: Pig longissimus dorsi muscle. Determination of slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast-twitch glycolytic (FG) fibers on a single cryosection using combine SDH histochemistry and immunolabeling with an anti-MHC-I (slow) isoform (41). A highly organized pattern and unique distribution of fibers composed of clusters of SO fibers, which are surrounded by FOG fibers and more external FG fibers can be observed

Because of its simplicity, muscle fiber classification into SO, FOG and FG is still widely used above all in studies in which basic information about contractile and metabolic properties of the muscles are required. To make the conventional enzyme-histochemical fiber typing more friendly to use, combined histochemical methods based on the successive staining of myosine ATPase and different metabolic enzymes such as SDH or NADH-TR on a single cryostat section have been developed (37, 38, 39). However, these techniques lead sometimes to unreliable fiber staining because of some incompatibilities in enzyme optimal conditions (40). More reliable results are usually obtained when combining successively metabolic enzyme staining and immunoistochemnical labeling of fibers (Fig. 2).

Patterns of muscle fiber type distribution in mammals

The distribution and proportions of fiber types vary between species and muscles. In most mammalian species, skeletal muscles exhibit a random spatial distribution of different fiber types. Fibers belonging to the same motor unit i.e. innervated by the same motorneurone, exhibit similar contractile and metabolic characteristics and are interspersed between fibers of other motor units. In small mammals, like rodents and lagomorphs, three fast MHC isoforms, -IIa, -IIx and -IIb are expressed in fast fibers (42, 43). On the contrary, MHC-IIa and -IIx isoforms are present in skeletal muscles of humans (44, 45), cats (46), dogs (25), cattle (47), goats (48), horses (49, 50) and brown bear (51).

The muscle fiber type composition depends on the specific function of a muscle and, furthermore, extends to species specific differences. From the comparison of fast MHC isoforms concentration between species it seems clear that the relative amount of MHC-IIb isoform decreases as body size increases, whereas that of MHC-IIa and -IIx increases. A possible explanation for such differences in muscle fiber composition between species could be that, that small animals with faster movements need faster twitch myofibers than larger animals which movements are slower. This hypothesis can explain why most large mammal species do not possess the fastest MHC-IIb isoform in their skeletal muscles. Such hypothesis was additionally confirmed in rabbit, where MHC-IIb isoform is more intensively expressed in young than adult animals. Decrease in the relative concentration of MHC-IIb isoform with increasing age possibly relates to the growth of the animal and changes in its locomotion pattern (24). MHC isoforms transformation associated with the process of growth was described also in large mammals, although they usually do not contain MHC-IIb isoform. In these species, the proportions of MHC-I and MHC-IIa myofibers increase, while that of MHC-IIx myofibers decreases during growth. In the early postnatal period the increased expression of MHC-I and -IIa isoforms is the consequence of a transition from developmental to adult MHC profile (52, 53). However, during later periods of growth some MHC-IIx myofibers obviously transform into MHC-IIa myofibers via hybrid MHC-IIa/x myofibers and into MHC I via hybrid MHC-I/IIa myofibers. Such transformations were observed in adolescent bears (51) and up to six years of age in different horse breeds (54, 55). With increasing age the percentage of hybrid fibers decreases, which supports their transitional role in muscle maturation. Taken together, the slower and more fatigue resistant characteristics of skeletal muscles with increasing age likely relate to a progressive adaptation to increasing body weight.

The lack of MHC-IIb isoform expression in most adult large mammals has been hypothesized to be related to body size and muscle fiber length. In large mammals the shortening of the fastest MHC-IIb isoform would produce such a high force that muscle could be injured (45). However, recent stud-

ies have shown the existence of all three fast MHC isoforms , including MHC-IIb, in adult pig longissimus (56) and llamas semitendinosus (57), which did not support hypothesis suggesting no expression of MHC-IIb in large mammals. In fact, gene coding for MHC-IIb isoform has even been discovered in humans; however, its expression in skeletal muscles remains to be confirmed (58). The reason why this isoform would be expressed only in pigs and llamas among large mammals is not known. Both species exhibit a so called type grouping distribution of the muscle fiber types with central clusters of MHC-I myofibers surrounded by MHC-IIa, then MHC-IIx and finally more external MHC-IIb myofibers (5, 56). In other species such fiber type grouping can be observed in relation to some neuromuscular disorders, whereas it is a normal spatial distribution in porcine skeletal muscle (59). Highly organized focal arrangement is supposed to be functionally relevant. Thus, central clusters of MHC-I myofibers would be most easily mobilized first for weak long-lasting contraction, whereas MHC-IIb fibers would be mobilized last for short-lasting forceful contraction. However, such a muscle fiber type distribution is not prerequisite, since most mammals exhibit random mosaic fiber distribution and are nevertheless fully functional. As well, it could be speculated that MHC-IIb isoform is expressed in some large glycolytic pig skeletal muscles as a result of intense selection for high muscularity and growth efficiency; however, further research is needed to test this hypothesis (60).

Correspondence between myofiber classification systems

It is well documented that maximal shortening velocity of muscle fibers expressing homologous MHC isoforms greatly decreases with increasing body size (61). Such functional diversity of the homologous fast MHC isoforms between species is likely related to different structural characteristics. This is probably one reason why MHC antibodies, which are usually raised against rat isoforms, can have diverse reactivity with homologous MHC in large mammals and why myosin ATPase histochemistry protocols must be adjusted to each animal species. Because of some important differences in the reactivity of MHC antibodies between species (Table 1), a set of different antibodies is usually used to avoid myofiber misclassification.

Table 1: Reactivity of commonly used antibodies raised against MHC isoforms in different species according to Smerdu at al. (6, 26, 51), Lefaucheur et al. (60) and Rivero et al. (49)

MHC isoforms \ Antibody		MHC-slow	A4.74	SC-71	F113, 15f4	BF-35	BF-F3
MHC-I	Rat	+	-	-	-	+	-
	Human	+	-	-	-	+	-
	Dog	+	-	-	-	+	-
	Bear	+	-	-	-	+	-
	Horse	+	-	-	-	+	-
	Pig	+	-	-	-	+	-
MHC-IIa	Rat	-	+	+	+	+	-
	Human	-	+	+	+	+	-
	Dog	-	+	+	+	+	-
	Bear	-	-	-	+	+	-
	Horse	-	+	+	+	+	-
	Pig	-	+	+	+	+	-
MHC-IIx	Rat	-	-	-	+	-	-
	Human	-	±	±	+	+ or -	-
	Dog	-	+	+	+	-	-
	Bear	-	+	+	+	-	-
	Horse	-	-	-	+	-	-
	Pig	-	-	±	+	-	-
MHC-IIb	Rat	-	-	-	-	+	+
	Human	-	-	-	+	+	-
	Dog	-	-	-	+	+	-
	Bear	-	-	-	+	+	-
	Horse	-	-	-	+	+	-
	Pig	-	-	-	+	+	+

(- = negative reaction, +/- = weak reaction, + = positive reaction)

The specificity of MHC-slow antibodies is unambiguous because they revealed type I myofibers in skeletal muscles of all species (25, 48, 49, 60), suggesting that the slow MHC-I isoform is highly conserved among species. On the opposite, fast MHC isoforms can show different antigenic properties between species. Thus, both A4.74 and SC-71 antibodies are specific to MHC-IIa isoform of rat, but cross-react with MHC-IIx in human, dog, pig, goat (26, 48, 60, 62, 63). In bear skeletal muscles both antibodies actually recognize MHC-IIx and not MHC-IIa isoform (51). This was confirmed with antibody BF-35, which reveals all MHC exept IIx in rat (42). Similar problems were

observed with the antibody F113.15F4, which recognizes MHC-IIa, -IIx and -IIb in rat, and only MHC-IIa and -IIx in dog and bear (25, 51). Some misclassification of myofibers between species also occurs using myosin ATPase histochemistry. The staining pattern of fibers depends upon the lability of myosin ATPase to pH preincubation and is related to the MHC isoforms content within a single myofiber. When two isoforms are expressed in the same myofiber, the staining pattern of the myosin ATPase is ambiguous and can lead to misclassification of the muscle fiber type, especially of fast type II fibers. In the past myosin ATPase based classification led to some contradictory reports on fast fiber sub-types in large mammals. In some studies of canine muscles only type IIA and IIC myofibers were found (64, 65, 66, 67). On the contrary, other authors claimed that type IIB myofibers are present even in dogs, although they were slightly less acid-labile than type IIB in other species (68). The immunohistochemical labeling of MHC isoforms demonstrated that strongly acid-stable subclass of canine fast fibers, which were dark after preincubation at pH 4.6 and would thus correspond to type IIB myofibers of other species, actually expressed MHC-IIa isoform, and the more acid labile sub-class, which were named as IIDog fibers (69, 70) actually corresponded to MHC-x fibers (26). Such integrated use of both myosin ATPase and immunohistochemical labeling of MHC isoforms demonstrated that type IIB fibers have been misclassified in numerous previous studies based upon traditional myosin ATPase histochemistry in other large mammals as well (71). Myosin ATPase can also lead to fiber misclassification because of partial denaturation of the enzyme. Thus, the rapid postmortem acidification combined with increased muscle temperature encountered in some glycolytic muscles of stress susceptible pigs can lead to irregular and altered myosin ATPase staining these PSE (pale, soft and exudative) muscles (Figure 3). In such case, the use of antibodies against MHC isoforms is a far more reliable technique to type myofibers (72).

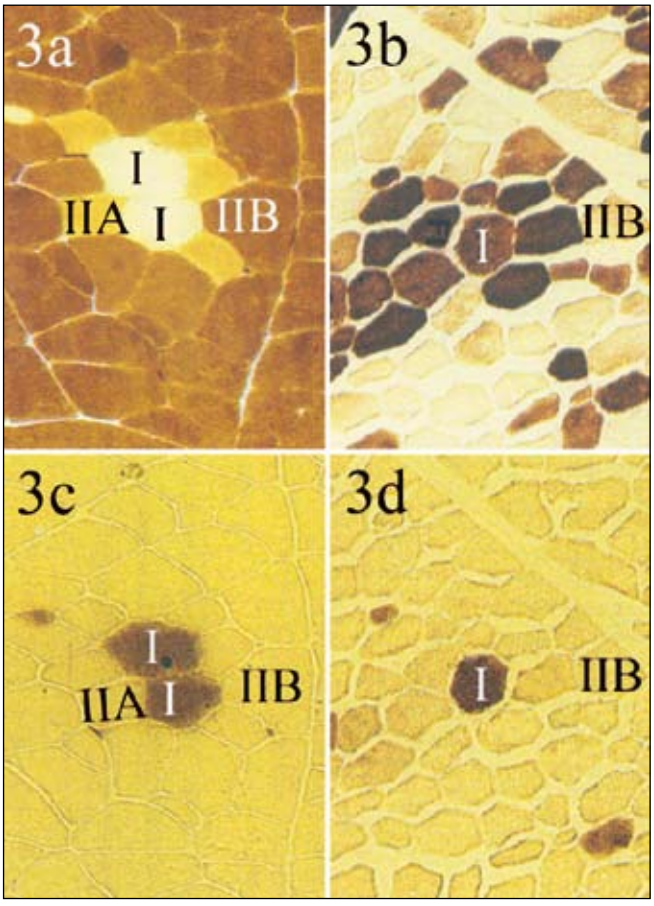

Figure 3: Alkali stable myosin ATPase activity (a, b) (73) and myosin ATPase activity pH 4.3 preincubation (c, d) of a normal (a, c) and PSE (b, d) pig longissimus dorsi muscle. Three different fiber types are distinctly recognized in normal muscle (I, IIA, IIB); whereas, the staining pattern of the alkali stable myosin ATPase is altered in PSE muscle. The PSE condition mostly inactivated the alkali stable myosin ATPase activity in peripheral fast-twich glycolytic IIB fibers

Conclusion

Big differences in muscle fiber type composition exist between muscles and species, and between individuals within species. It is well documented that skeletal muscles is a highly adaptable tissue which can be influenced by many intrinsic and extrinsic factors, such as age, altered neuromuscular activity and mechanical loading. The principal methods to type myofibers on the tissue cryosection are the immunohistochemical detection of MHC isoforms and the myosin ATPase and metabolic enzyme histochemistry. Overall, it must be stressed that immunohistochemical and enzyme histochemical classifications are not always fully interchangeable between and even within species, suggesting that different techniques often have to be combined to get a reliable myofiber typing.

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ENCIMSKO-IMUNOHISTOKEMIČNI VIDIKI RAZVRŠČANJA TIPOV MIŠIČNIH VLAKEN PRI SESALCIH

G. Fazarinc

Povzetek: Skeletne mišice so pri sesalcih tkivo, ki ga je v telesu največ in je tudi najbolj prilagodljivo. Sestojijo iz mišičnih vlaken, ki se razlikujejo po vsebnosti strukturnih proteinov kot tudi po aktivnosti presnovnih encimov. Zato številčni deleži tipov vlaken v mišici določajo njene morfološke in funkcionalne značilnosti. V članku so predstavljene osnovne encimsko-imunohistokemične tehnike, na osnovi katerih prepoznavamo značilnosti posameznih tipov mišičnih vlaken kot tudi njihovo raznovrstnost v skeletnih mišicah različnih živalskih vrst. Na tkivnih rezinah razvrščamo mišična vlakna na osnovi imunohistokemičnega določanja vsebnosti izoform težkih miozinskih verig (MHC), aktivnosti miozinske ATP-aze in aktivnosti presnovnih encimov. V somatskih skeletnih mišicah manjših sesalcev so dokazane štiri različne izoforme težkih miozinskih verig (MHC-I, -IIa, -IIx in –IIb). Glede na vsebnost izoforme raste hitrost krčenja mišičnih vlaken v naslednjem zaporedju MHC: -I < -IIa < -IIx < -IIb. V t. i. hibridnih vlaknih sta izraženi dve izoformi MHC in sta pokazatelj preobrazbe mišičnih vlaken. Na osnovi aktivnosti miozinske ATP-aze vlakna razvrščamo v tipe I, IIA, IIB in IIC. Vlakna tipa IIC predstavljajo prehodni tip med vlakni MHC-I in MHC-IIa. Mišice pri večini velikih sesalcev ne vsebujejo najhitrejše MHC-IIb izoforme, vendar pa so zadnje študije pokazale prisotnost vseh treh hitrih MHC izoform tudi pri domačem prašiču in lami. Zrkelne, grlne in žvekalne mišice, ki se tako funkcijsko kot razvojno razlikujejo od somatskih, vsebujejo tudi t. i. ekstraokularno (MHC-ekstraokularna) oz. mastikatorno (MHC-m) izoformo, med razvojem in regeneracijo pa so v mišicah prisotne še razvojne izoforme (MHC-embrionalna, MHC-neonatalna). Počasna MHC-I vlakna kažejo veliko oksidativno presnovno zmožnost, v hitrih MHC-II vlaknih pa je aktivnost presnovnih encimov zelo različna. Na osnovi krčljivostnih lastnosti mišičnih vlaken in ob upoštevanju njihove presnovne aktivnosti jih lahko razvrstimo v tri osnovne tipe: počasi krčljiva oksidativna (SO), hitro krčljiva oksidativno-glikolitična (FOG) in hitro krčljiva glikolitična (FG). Razvrščanje vlaken na osnovi samo ene od opisanih metod je večkrat problematično, zato je za natančno in zanesljivo določitev lastnosti mišičnih vlaken potrebno sočasno uporabiti različne encimsko-imunohistokemične tehnike.

Ključne besede: skeletna mišica; težka miozinska veriga; tip mišičnega vlakna; histokemija

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Original Research Paper

INFLUENCE OF GRADUAL CHANGE IN FEED, USE OF ACIDIFIER AND PREBIOTIC ON RABBITS IN THE PERIOD OF WEANING

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Summary: To establish the possibility of improvement of rabbit production results, the use of feed acidifier, supplements to feed and gradual change from feed for does to feed for weanlings was investigated. Ten days before parturition 3 groups of 15 pregnant does each were formed. They were fed feed containing coccidiostatic drug Robenidin (1 mg/kg). To the first two groups acidifier Acid Pac 4 Way (2 g/l) was administered in drinking watter. Between day 22 and 30 of weanlings age gradual change from feed for does to feed for weanling rabbits, that contained prebiotic Bio Mos (2 g/kg), was carried out in the first group. The second group got the same feed but there was no gradual change of feed. In the third group gradual change of feed was performed, but for weaned rabbits only basic feed without supplements was used.

The results of parturitions, feed consumption, number and weight of weaned rabbits to the 40th day of age and losses of does and their youngs were registered. Statistically significant difference ($\bar{x}$ ±SE) in the number of equalized and weaned rabbits were stated between 2nd (7.3±0.3, 6.7±1.91) and 3rd (8.7±0.21, 9.5±1.24) group (P<0.05). At weaning the greatest average weight of youngs was found in the 1st group (640.7±19.29 g) and at the age of 40 days in the 2nd group (934.1±10.41 g). The results showed that feed supplements can contribute to better results in intensive rabbit production.

Key words: animals, feeding; feed, additives; oligosaharides; animals, suckling; rabbits

Introduction

In the last decades, the performance of intensive rabbit production improved a lot, due to the development of specialised strains (hybrids), increasing use of artificial insemination, adapted diets and management rationalisation. However, mortality of youngs before and after weaning is still very high and mounts in total to nearly 25%. The major part of these losses is due to diarrhoea, whose etiology is multifactorial (1). The nutrition requirements of a rabbit derive from physiological processes of digestion. Being herbivorous a rabbit needs crude fibres, which assure normal physiological activity of digestion and reduce the appearance of metabolic disorders. Selective separation of particles inside ileo-cecal region and the ability of repeated utilization of soft faeces by cecotrophy represent two particularities of rabbit digestion with specific influence on digestive processes (1, 2).

For economical reasons in the intensive breeding of rabbits pelleted feeds are used (3). Due to small number of rabbit farms in Slovenia and correspondingly small demand for rabbit feeds, their production still represents a lateral program of feed industry. Customary three types of feed are used (for does, for weanling rabbits and for animals in fattening), despite the known fact that different categories of rabbits cannot be fed the same feed by regulating its amount in correspondence to the category (4).

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Actually the nutrition requirements of young rabbits after weaning and their mothers are antagonistic. Does have great demands for energy while the use of feed with low starch and high fibres content before weaning benefits the health status of weaned youngs. By classical technology of feeding young rabbits are fed together with their mothers from the same vessel and get special feed for weanling animals only after weaning. Frequently transition from milk to solid pelleted feed represents greater stress than weaning itself (1,5). Main problem of growing rabbits nutrition is thus securing of physiological balance between adequate provision of nutritional substances and avoiding of metabolic disturbances related to disharmonies of this provision. Symptoms of such digressions are in principle diarrhoea and higher mortality, especially in the period of weaning, causing great economic losses (6).

Besides the improvement of technological procedure of young rabbits transition from suckling period to the period after weaning, in intensive rabbit production different supplements (enzymes, nutritive antibiotics, flavours, probiotics, acidifiers etc.), helping in diminishing the stress, are used in feed or water. Because of illness complexity and lack of special products for rabbits the influence on microbial population of guts is in principle less successful. Anyhow some researches show positive effect of probiotics and acidifiers supplementation on rabbit production, feed conversion and on reduction of enteritis frequency (5,7). It is also considered that young rabbits with higher body weight by weaning show lower sensibility to weaning stress (1).

The presented research was performed to study the possibilities for diminishing of digestion disturbances in young rabbits by corresponding mode of feeding with the aim of lowering their mortality and achieving higher body weight at weaning.

Material and methods

Animals

The experiment was carried out in facilities for parent stock and for fattening rabbits of a bigger rabbit farm. All the technological procedures except feeding, where daily consumption of feed was controlled, were standard for the farm. The experiment included 45 pregnant, clinically healthy females of New Zealand - Californian crossbreed, from 38 do 90 weeks old, weighing between 3700 g and 5230 g. The experiment started ten days before parturition when the does were divided into three groups of 15 animals each. At the day 13 after delivery the does were inseminated and 18 days later the fertility control was carried out.

After delivery the offsprings were left in the groups of their mothers and were controlled until the day 10 after weaning – to the average age of 40 days. Two days after the last delivery in the group, the litters inside the group were equalized by size and number of kits, considering the principle that the litter must be composed from young rabbits with the same outliving ability and that weak litters belong to the does with higher milk production. In the case of doe's loss, the kits were divided among other litters of the same group.

Weanling rabbits were transferred to fattening stall at the average age of 30 days. For the first time they were weighed on the day of equalization and later at average age of 10, 17, 24, 31, and 40 days.

Health status and mortality of animals were controlled during the whole experiment.

Feed and feeding

The pelleted feed was prepared in the farm owned feed factory following the standard prescription used in the farm. The consumption was not limited and the composition of basic feed was as follows:

- feed for does contained: 37% alfalfa, 20% wheat bran, 15% oats, 11% sunflower meal, 8% dried beet slices, 4.25% barley, 1.5% vegetable oil, 1% pinotan (lignin sulphate as pellet binder), 0.7% dicalcium phosphate, 0.25% salt, 0.2% lisin, 0.1% alimet (liquid metionin - 88%), 1% premix for does and 1.4g/kg of natural tannin extract (extract from chestnut wood),
- feed for weanling rabbits: 37% alfalfa, 25% wheat bran, 23.7% oats, 6% dried beet slices, 5% soybean meal, 1% vegetable oil, 1% pinotan, 0.2% salt, 0.1% alimet, 1% premix for weanling rabbits and 2.0g/kg of natural tannin extract (extract from chestnut wood).

Regarding the program of the trial, the basic feeds were supplemented with Robenidin (coccidiastatic) or Bio Mos (Alltech Inc.) - beer leaven from yeast *Sacharomyces cerevisae* containing mannooligosaccharides, structural parts of yeast cell wall.

The samples of feeds were analysed at The Institute for Hygiene and Pathology of Animal Nutrition

of Veterinary Faculty in Ljubljana. Chemical composition was determinated by Weende analyses (8), whereas macro- and microelements: calcium, magnesium, sodium, potassium (9), manganese, zinc, copper and iron (10) were determined by atomic absorption spectrometry and phosphorus spectrophotometricaly (11). The microbiological quality of feeds was established in accordance with the procedure by Schmidt et al. (12). The number of grown colonies of mesofilic aerobic bacteria, moulds and yeasts was ascertained and expressed in colony forming units per gram feed.

For the gradual change from feed for does to feed for weanling rabbits, carried out between 22nd and 30th day of young rabbits· age, mixtures of both feeds were prepared and fed in appointed proportions. At the day 22nd and 23rd of the young rabbits age mothers and their offsprings were fed 80% of feed for does and 20% of feed for weanling rabbits. Next 3 days both feeds were mixed in proportion 50:50. Afterwards, up to the 29th day the mixture contained 20% of feed for does and 80% of feed for weanling rabbits. At the day of weaning (30th day of age) they got only feed for weanling rabbits.

The does returned to their own feed after three days. At the first day they got 80% of feed for weanling rabbits and 20% of feed for does, at the second day 50% of feed for weanling rabbits and 50% of feed for does and at the third day only 20% of feed for weanling rabbits and 80% of feed for does. At the fourth day they were fed only feed for does.

During gradual change of feed does got feed for weanling rabbits with no coccidiostatic drug. Therefore their faeces were coprologically controlled 3 times: at delivery, 3 weeks after delivery and 3 days after weaning. Collective faeces sample of each group was taken from ten places of the floor under cages.

Acidifier in the drinking water

In the 1st and the 2nd group of animals acidifier Acid Pac 4 Way (Alltech Inc.), in concentration of 2 g/l, was supplemented in fresh drinking water, which was permanently available to does and their kits until weaning. Afterwards, weaned rabbits got water with no acidifier.

Basic feeds, supplements and feeding technology used in the experiment are presented in table 1.

Table 1: Scheme of feeds, supplements and feeding technology used in the experiment

group	feed supplements		Acid Pac 4 Way in water (only for does)	gradual change from feed for does to feed for weanling rabbits
	feed for does	feed for weanling rabbits		
1	feed A	feed 1	YES	YES
2	feed A	feed 1	YES	NO
3	feed A	feed 2	NO	YES

Legend: feed A: Basic feed for does + coccidiostatic Robenidin (1mg/kg)
feed 1 : Basic feed for weanling rabbits + prebiotic Bio Mos (2 g/kg)
feed 2 : Basic feed for weanling rabbits

Statistical methods

The results were statistically evaluated by one way ANOVA, followed by posthoc Scheffe test. All data were analysed using SPSS (Statistical Package for Social Sciences - Version 12, November 2003) software package.

Results

Feed analyses

The results of chemical analyses of feeds (table 2) were estimated regarding producer's declaration. Considering maximum permitted deviations and measurement uncertainty of used methods no digression from declared values was found.

Consumption of feed

In the first fifteen days after delivery feed was available only to does and afterwards also to their offsprings. No statistical significant difference between groups was observed regarding average consumption of feed (does + litter) from delivery to weaning. The lowest average consumption was observed in the second group (440.6 ± 185.1 g feed per day) and the highest in the first group (486.9 ± 199.3 g feed per day). The highest average day consumption of feed from weaning to the 40th day of age, calculated on single weaned rabbit, was found in the 1st group (table 3).

Table 2: Analyzed composition and microbial content of diets for does and weanling rabbits

analysis	feed for does	feed for weanling rabbits	
		Bio Mos	no supplement
dry matter (g/kg)	892.1	904.3	895.2
humidity (g/kg)	107.9	95.7	104.8
crude proteins (g/kg)	159.2	150.6	151.1
crude fibres (g/kg)	163.5	150.7	149.6
crude fat (g/kg)	30.0	28.0	30.0
ash (g/kg)	72.8	71.0	70.7
nitrogen free extract NFE (g/kg)	466.6	504.0	493.8
starch (g/kg)	173.9	195.6	163.0
calcium (g/kg)	8.0	7.0	6.8
phosphorus (g/kg)	6.1	4.7	4.6
pothassium (g/kg)	11.3	11.0	10.6
sodium (g/kg)	1.7	2.0	1.8
magnesium (g/kg)	3.0	2.9	2.8
zinc (mg/kg)	157.0	129.7	106.3
copper (mg/kg)	28.7	33.0	22.2
manganesse (mg/kg)	177.4	144.0	125.8
iron (mg/kg)	519.0	570.8	556.6
chlorides (g/kg)	4.4	5.2	5.3
yeasts (v 1000/g)	0	0	0
total number of moulds (v 1000/g)	0	0	1.0
Aspergillus spp. (v 1000/g)	0	0	1.0

Table 3: Average day consumption of feed from weaning to the 40th day of age

	group 1		group 2		group 3		P		
	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	1 vs.2	1 vs.3	2 vs.3
consumption of feed (g/weanling)	108	105.4 ± 9.76	98	95.9 ± 11.66	97	84.7 ± 6.58	0.732	0.869	0.782

Legend: n = nr. of 40 days old rabbits; $\bar{x}$ = average day consumption of feed; SE = standard error

The highest feed conversion in the first 10 days after weaning was also stated in the 1st group (3.7 kg of feed per 1 kg of weight gain) while the lowest in the 3rd group (2.8). Feed conversion of the 2nd group was 3.1 kg of feed per 1 kg of weight gain.

Deliveries

The deliveries took place from 30th to 34th day of gestation. Out of 45, 44 does delivered, so the 1st group was formed only from 14 litters, one less than the other two groups. The greatest number of live born, equalized and weaned rabbits was in the 3rd group (table 4).

Table 4: Number of live born, equalized and weaned rabbits in different groups

	group 1		group 2		group 3		P		
	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	1 vs.2	1 vs.3	2 vs.3
live-born	14	8.6 ± 0.99	15	8.3 ± 0.89	15	9.3 ± 1.01	1.00	0.999	0.991
still-born	14	1.8 ± 0.96	15	0.7 ± 0.66	15	0.5 ± 0.53	0.912	0.865	1.000
equalized	14	8.1 ± 0.34	15	7.3 ± 0.30	15	8.7 ± 0.21	0.444	0.884	0.034
nr. of weanling	13	7.3 ± 3.35	15	6.7 ± 1.91	12	9.5 ± 1.24	0.971	0.093	0.008

Legend: n = number of animals in the group; $\bar{x}$ = average body weight; SE = standard error

Statistical comparisation between 2nd and 3rd group showed significantly higher number of equalized rabbits (P = 0.034) and weaned rabbits / doe in the 3rd group (P = 0.008).

Body weight of young rabbits

Average weight of young rabbits by equalization and at the 10th day of age was similar in all three groups. At the 17th day of age retardation of weight gain was observed in rabbits of the 2nd and especially of the 3rd group (table 5). With exception of rabbits from the 3rd group, the average body weight of rabbits at weaning exceeded 600 g. At the end of experiment (at the age of 40 days) the highest average body weight of rabbits was in the 2nd and the lowest in the 3rd group.

Table 5: Average body weight and the number of young rabbits in groups from equalization to weaning

body weight of young rabbits (g)	group 1		group 2		group 3		P		
	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	1 vs.2	1 vs.3	2 vs.3
at equalization	114	93.3 ± 5.46	109	91.9 ± 4.60	130	90.5 ± 5.22	1.000	0.999	1.000
10th day of age	111	192.2 ± 8.91	104	187.0 ± 6.49	127	171.1 ± 9.26	1.000	0.640	0.813
17th day of age	111	291.1 ± 12.08	102	264.0 ± 10.84	126	254.7 ± 11.89	0.729	0.461	0.997
24th day of age	111	404.3 ± 21.68	100	395.1 ± 11.64	120	351.1 ± 13.77	0.999	0.382	0.583
at weaning	110	640.7 ± 19.29	100	627.0 ± 21.01	114	578.8 ± 28.00	0.999	0.909	0.999
40th day of age	108	922.7 ± 16.36	98	934.1 ± 10.41	97	881.9 ± 27.28	1.000	0.884	0.772

Health status control

Mortality observed during the experiment in all groups and categories of animals could be considered as the consequence of technological reasons. Five casualties among does were due to pneumonia and endometritis and the reason for mortality at young rabbits was mainly diarrhoea.

Loss of kits from equalization to weaning was the greatest in the 3rd and the smallest in the 1st group (figure 1). After weaning the mortality in the 3rd group was still increasing while in the 1st and the 2nd group it diminished to the practically equal amount (1.8% and 2%).

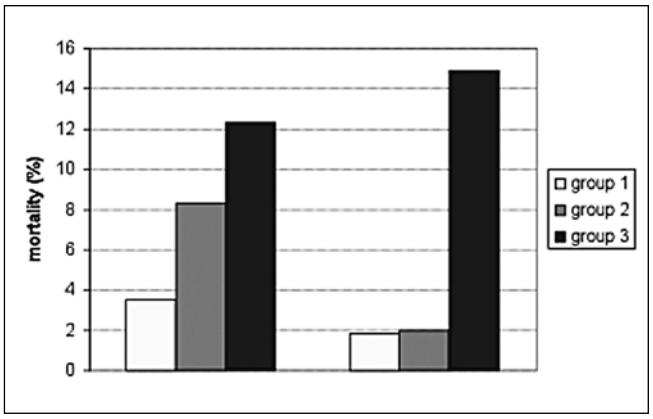

Figure 1: Percentage of mortality from equalization to weaning and from weaning to the age of 40 days

Coprological examinations of faeces

The contamination of does with coccidia ooccysts and eggs of other parasites is presented in table 6.

Table 6: Results of coprological examinations of does faeces

group	results of coprological examinations		
	on the day of delivery	21st day of litter age	three days after weaning
1	0	Passalurus ambigus +	Passalurus ambigus +
2	Passalurus ambigus +	Passalurus ambigus +	Passalurus ambigus +
3	single oocysts	Passalurus ambigus +	Passalurus ambigus +

In the facilities for parents stock no clinical case of coccidiosis was stated. The presence of coccidia was confirmed only in the 3rd group, but the low number of stated coccidia prevented their determination. In the groups with gradual change of feed (group 1 and 3) the number of coccidia oocysts was not increased.

Discussion

Concerning the results of different management of rabbit feeding, there was no significant difference between the three experimental groups regarding body weight of weaned rabbits and average day consumption of feed from weaning to the 40th day of age, representing the essential data of the experiment. The average number of weaned rabbits was statistically significantly higher in 3rd group in comparison with 2nd, but the mortality in this group was during the experiment the highest and the average body weights of rabbits were the lowest. Gradual change from feed for does to feed for weanling rabbits did not improve production results, but the positive effect of prebiotic and acidifier supplementation was clearly obvious from higher body weight and lower mortality in the 1st and 2nd groups where they were used.

Feed for does and weanling rabbits that was normally used on the farm was applied in the experiment. Especially the feed for weanling rabbits was problematic due to provision of rough material with higher crude protein and crude fiber content. The content of starch was also too high, which was obvious from the analyses of feed for does and feed for weanling animals where similar values of starch and other nutritive substances were detected (table 2). The comparison of crude fibre with normative values reviewed by Kermauner (13) showed their lack in the feed for weanling rabbits (150.7 and 149.6 vs. ≥ 155 g/kg). Problems of crude fibres, crude proteins and starch content in rabbit feeds are known elsewhere in the world, but the conclusions of different authors involved in that problem are not the same (14, 15, 16, 17, 18).

The lowest mortality to weaning (3.5%) in our experiment took place in the 1st group. In comparison to the 2nd group the breeding results in this group over the first ten days were better despite the fact that no other difference was stated until the introduction of gradual change of feed (21st day of age). The rabbits from the 1st group had also the highest body weight at weaning but later, in the period to the 40th day of age, their breeding results were lower from those of the 2nd group. Such results are hard to evaluate. However, great variety of results is obvious also from other experiments on rabbits. Di Meo et al. (19) investigated the effect of two different solid feeds during suckling on productive performance and caeccal content characteristics of rabbits at weaning (28 days). From day 16th, the first group was administered a commercial weaning diet and to the others was given the same feed as to their mothers. After weaning, the rabbits from both groups received *ad libidum* diet for weanlings, and later a finisher diet. The differences between body weight of mentioned groups were statistically significant only in the 1st week after weaning. The consumption of feed and production rate in the first group was better, which

was explained by feeding feed for weaned animals in this group before weaning. Feed intake before weaning has no greater influence on hint gut fermentation but contributes to easier transition from milk to pelleted feed thus diminishing risks of nutritional disturbances. Their opinion was confirmed also by Gidenne and Fortun - Lamothe (20), who investigated different technological procedures of weaning. They emphasize the benefit of feeding young rabbits specifical feed if this feeding is performed between 28th and 35th day of age. In the opposite case suitable technological solutions enabling compromise between nutritional needs of mothers and their offsprings must be taken in consideration (20, 21). However the effect of gradual change of feed performed in our experiment was most probably not clearly obvious because of great composition similarity of feed for does and feed for weaned rabbits. The mortality of young rabbits was the greatest in the 3rd group. Following the results of research including 850 French rabbit farms, Gidenne and Fortun-Lamothe (20) stated that 30% of mortality from birth to slaughtering is not rare. In our experiment the greatest mortality to weaning (12.3%) and 14.9% from weaning to the 40th day of age was found in the 3rd group where only gradual change from feed for does to feed for weanling rabbits was used. In the same group the greatest appearance of digestive disturbances was observed, which resulted in average body weight at weaning lower than 600 g, which was surpassed in both other groups.

Comparison of the 3rd group to other two groups also showed that prebiotic (Bio Mos) supplementation can contribute to better results in intensive rabbit production. Probiotics, containing bacteria from *Bacillus* species or different yeasts, were found to be useful in the nutrition of rabbits also by other authors (22). Use of probiotic Paciflor contributed to significantly better growth rate during fattening and lower mortality in stress situations such as high temperature and low weaning weight (23). Similar results were obtained by Dupperay and Roberton (24) and Szabo-Lacza et al. (25, 26). On the contrary Maertens et al. (27), using the same probiotic, did not report statistically better growth results during fattening.

In some researches Bio Mos (Alltech Inc.) was stated to be a perfect supplement to feed for rabbits (28). Supplementation of 2 kg/1000kg of feed lowered mortality caused by enteritis (29). Regarding Tibor et al. (30) in rabbits supplemented with Bio Mos daily weight gain was 9.2% and the body weight at the end of experiment 5.5% higher. Kocher et al. (31) performed the analysis of 20 experiments in which feed for weanling rabbits was supplemented with antibiotics, Bio Mos or contained no supplements. By use of Bio Mos better growth results ($P = 0.001$) and feed conversion was observed in comparison with control without Bio Mos supplementation. In 19 experiments lower mortality was also stated.

Benefits of organic acidifier Acid Pac 4 Way (Alltech Inc.) supplementation in the feed for young rabbits before and after weaning were also described. Its application applenish the production of gastric acids in the critical period of weaning, when a young rabbit has no ability for maintaining of correspondingly low Ph, which represents an important barrier against invasion of pathogenic bacteria (32). Cheeke et al. (33) also stated the beneficial effects of Lacto-sacc and Acid Pac 4 Way supplementation on breeding results, microbial digestion in caecum, weight at weaning and diminishing of mortality. In our experiment the supplementation of acidifier in drinking water for does started 10 days before parturitions so the results of deliveries were, due to short period of its use, most probably not influenced. Anyhow the beneficial effect of acidifier supplementation occurred from the 16th day of their age, when they started to take their mothers' feed.

After weaning the best results of weight gain was found in the 2nd and the worst in the 1st group. Unfortunately in our experiment following the animal body weight till slaughtering was not possible owing to production technology of the farm.

Although no significant difference was observed between the groups, the results lead to conclusion that supplementation of Bio Mos (Alltech Inc.) in feed for weanling rabbits and use of acidifier Acid Pac 4 Way in drinking water effected better production results.

Gidenne and Fortun-Lamothe (20) stated that momentary change of feed during lactation reduces feed intake of does and has drastic consequences on milk production. Following their conclusions gradual change from feed for does to the feed for weanling rabbits should improve the technology of rabbit feeding, which was also the reason why it was introduced in our experiment. In our opinion such mode of feeding can be successfully introduced in production technologies where feeding of mothers and their kits cannot be separated.

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VPLIV POSTOPNEGA PREHODA KRMLJENJA, ZAKISOVALCA IN PREBIOTIKA PRI KUNCIH V OBDOBJU ODSTAVITVE

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Povzetek: V poskusu smo poskušali ugotoviti ali lahko s kombiniranjem zakisovalca, krmnih dodatkov in postopnega prehoda s krme za samice na krmo za odstavljence izboljšamo rejske rezultate pri kuncih. Deset dni pred kotitvami smo oblikovali 3 skupine po 15 brejih samic. Krmili smo jih s krmo, ki je vsebovala kokcidiostatik Robenidin (1 mg/kg). V vodi sta prva in druga skupina samic dobivali zakisovalec Acid Pac 4 Way (2 g/l). V skupini 1 smo od 22. do 30. dne starosti mladičev izvedli postopni prehod s krme za samice na krmo za odstavljence, ki je vsebovala prebiotik Bio Mos (2 g/kg). V skupini 2 postopnega prehoda ni bilo, krma za odstavljence pa je bila enaka kot v skupini 1. V skupini 3 je bil izveden postopen prehod, krma za odstavljence pa ni vsebovala nobenih dodatkov. Spremljali smo rezultate kotitev, porabo krme, število in težo odstavljenih mladičev do starosti 40 dni ter izgube med samicami in mladiči. Statistično značilna razlika ($\bar{x}$±SE) v številu izenačenih in odstavljenih kuncev je bila ugotovljena med drugo (7,3±0.3, 6,7±1,91) in tretjo (8,7±0,21, 9,5±1,24) skupino ($P<0,05$). Ob odstavitvi so bili najtežji kunci v 1. skupini (640,7 ± 19,29 g), pri starosti 40 dni pa kunci v 2. skupini (934,1 ± 10,41 g). Rezultati kažejo, da krmni dodatki v intenzivni reji kuncev lahko prispevajo k boljšim rejskim rezultatom.

Ključne besede: živali, prehrana; hrana, dodatki; oligosaharidi; živali, sesne; kunci

KEMOMED

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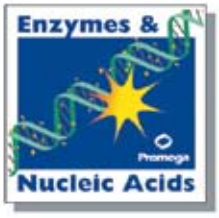

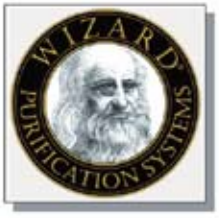

IZDELKI ZA MOLEKULARNO BIOLOGIJO

DOKUMENTACIJA IN ANALIZA GELOV

PLASTIKA ZA CELIČNE KULTURE

ELGA LABWATER

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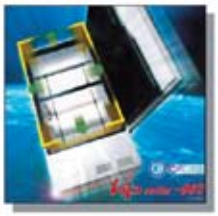
Invitrogen life technologies



ČISTA VODA ZA LABORATORIJ

SKRINJE IN HLADILNIKI

CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA

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ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL

HPLC in GC POTROŠNI MATERIAL

INSTRUCTIONS FOR AUTHORS

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Contributions should be written in English and should not exceed 12 pages (27 lines per page, approx. 75 characters per line). They should be submitted electronically (preferably to E-mail address, slovetres@vf.uni-lj.si), written in any word processor for Windows. Authors are requested to provide names of three potential reviewers. The text should be double spaced and the lines should be numbered on the left-hand side. The margin on the left-hand side of the page should be 4 cm.

The front page of a manuscript should start with the title, followed by the name and surname of the author(s). If there is more than one author, their names should be separated by commas. The next line ('Addresses of authors:') should contain the authors' full names and addresses (institution, street and number, postcode and place) after the colon. All the given data should be separated by commas. The name, address and E-mail and/or phone number of the corresponding author should be written in the next line.

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Under 'Keywords:' (after the colon), keywords should be given. Individual words or word combinations should be separated by semicolons. Scientific papers and papers which present the author's research and findings should also include the following obligatory headings assigned by the author to appropriate parts of the text: Introduction, Materials and methods, Results, Discussion, and References. Review articles should consist of an introduction, sections logically titled according to the content, and references. Information on fund-providers and other matters important for the paper (e.g. technical assistance) should be supplied under 'Acknowledgements', which should be placed before the references. Figure legends should follow the references.

Tables, graphs and diagrams should be logically incorporated in the text file. Original photographs or drawings should be sent as separate files in bmp, jpg or tif format. They should be referred to by type and using Arabic numerals (e.g. Table 1:, Figure 1:, etc.). The colon should be followed by the text or title. All references cited in the text should appear in the References. They should be numbered in the text in the order in which they appear, marked with Arabic numerals placed in parenthesis. The first reference in the text should determine the number and order of the respective source in the References. If the author refers again to a source which has already been used in the text, he should cite the number the source had when it was referred to for the first time. Only works which have been published or are available to the public in any other way may be referred to. Unpublished data, unpublished lectures, personal communications and similar should be mentioned in the references or footnotes at the end of the page on which they appear. Sources in the References should be listed in the order in which they appear in the text. If the source referred to was written by six authors or less, all of them should be cited; in the case of seven or more authors, only the first three should be cited, followed by 'et al.'.

Any errata should be submitted to the editor-in-chief in good time after publication so that they may be published in the next issue.

Examples of references

Book: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Chapterorar ticle in a book: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Article in a journal or newspaper: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Article in proceedings of a meeting or symposium: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

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Slovenski veterinarski zbornik (Slovenian Veterinary Research) objavlja izvirne prispevke, ki še niso bili objavljeni oz. poslani v objavo drugam. Za vse navedbe v prispevkih so odgovorni avtorji. Uredniška politika obsega publiciranje znanstvenih člankov, preglednih znanstvenih člankov, strokovnih člankov, povzetkov disertacij in drugih prispevkov, kot so kritične presoje o vsebini razprav, objavljenih v zborniku, kratke znanstvene prispevke, pisma uredniku in drugo. Avtorji pošljejo prispevke na naslov uredništva. Glavni urednik pregleda vse prispevke. Za vse članke je obvezna strokovna recenzija, za katero poskrbi uredništvo.

Prispevki naj bodo napisani v angleškem jeziku, z naslovom, povzetkom in ključnimi besedami tudi v slovenščini. Obsegajo naj največ 12 strani, kar pomeni 27 vrstic na stran s približno 75 znaki v vrstici. Prispevki naj bodo poslani v elektronski obliki v katerem koli urejevalniku besedil za okensko okolje. Zaželjena je uporaba elektronske pošte (slovetres@vf.uni-lj.si) in avtorji naj predlagajo tri možne recenzente. Besedilo naj ima dvojni razmik med vrsticami, pri čemer naj bodo vrstice na levi strani oštevilčene. Besedilo naj bo na levi strani od roba oddaljeno 4 cm.

Naslovna stran prispevkov se začne z naslovom, sledi ime in priimek avtorja. Kadar je avtorjev več, jih ločimo z vejicami. V naslednjih vrsticah je v rubriki Addresses of authors: za dvopičjem treba navesti polno ime in priimek ter naslov(e) avtorja(ev), tj. ustanovo, ulico s hišno številko, pošto in kraj. Vse navedene podatke ločujejo vejice. Sledi vrstica, kjer je treba navesti ime ter elektronski (E-mail:) in poštni naslov ter telefonsko številko (Phone:) odgovornega avtorja.

Sledi besedilo povzetka Summary v obsegu 200 do 300 besed. V naslednji rubriki Key words: se za dvopičjem navedejo ključne besede. Posamezne besede ali sklopi besed morajo biti ločeni s podpičjem.

Znanstveni članki in tisti, ki so prikaz lastnih raziskav in dognanj, morajo vsebovati še naslednje obvezne rubrike, s katerimi avtor sam naslovi ustrezne dele besedila v prispevku: Introduction, Material and methods, Results, Discussion in References. Pregledni članki naj vsebujejo uvod, poglavja, ki so glede na vsebino smiselno naslovljena, in literaturo. Podatke o financerjih ali drugih zadevah, pomembnih za prispevek, npr. o tehnični pomoči, avtorji navedejo v rubriki Acknowledgements, ki se uvrsti pred rubriko References. Za rubriko References sledijo spremna besedila k slikam.

Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

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Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

Načini citiranja

Knjiga: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Poglavje ali prispevek v knjigi: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Članek iz revije ali časopisa: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Članek iz zbornika referatov: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

Slov Vet Res 2009; 46 (2)

Review Papers

Ornik D, Čadonič-Špelič V. Records on the use of animals in experiments in the Republic of Slovenia and in other EU member states within 15-years period 47
Fazarinc G. Enzyme-immunohistochemical aspects of muscle fiber type classification in mammals 61

Original Research Paper

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