ISSN 1580-4003

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Slov Vet Res 2011; 48 (1): 1-34

Volume 48 1

Slov Vet Res • Ljubljana • 2011 • Volume 48 • Number 1 • 1-34

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Volume 48 1

Slov Vet Res • Ljubljana • 2011 • Volume 48 • Number 1 • 1-34

The Scientific Journal of the Veterinary Faculty University of Ljubljana

SLOVENIAN VETERINARY RESEARCH
SLOVENSKI VETERINARSKI ZBORNIK

Previously: RESEARCH REPORTS OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA
Prej: ZBORNIK VETERINARSKE FAKULTETE UNIVERZA V LJUBLJANI

4 issues per year / izhaja štirikrat letno

Address: Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia
Naslov: Veterinarska fakulteta, Gerbičeva 60, 1000 Ljubljana, Slovenija
Tel.: +386 (0)1 47 79 100, 47 79 129, Fax: +386 (0)1 28 32 243
E-mail: slovetres@vf.uni-lj.si

Sponsored by the Slovenian Research Agency
Sofinancira: Agencija za raziskovalno dejavnost Republike Slovenije

ISSN 1580-4003
Printed by / tisk: Birografika Bori d.o.o., Ljubljana
Indexed in / indeksirano v: Agris, Biomedicina Slovenica, CAB Abstracts, IVSI
Urlich's International Periodicals Directory, Science Citation Index Expanded,
Journal Citation Reports/Science Edition
http://www.slovetres.si/

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Slov Vet Res 2011; 48 (1)

Slov Vet Res 2011; 48 (1): 5-14
UDC 577.2:616.8:616.714.1-006:599.323.4

Review Article

A RODENT BRAIN ORTHOTOPIC MODEL TO STUDY HUMAN MALIGNANT GLIOMA

Uroš Rajčević

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

E-mail: uros.rajcevic@nib.si

Summary: Glioblastomas are fatal brain tumors. They have a relatively low incidence, but the fact that regardless of the advances in therapy there hasn't been a major progress in survival over several decades urges the scientists to search for novel diagnostic tools, anti-tumor drug targets and therapies. It appears that our inability to successfully treat brain cancer mostly stems from the lack of understanding of the underlying brain tumor biology. Many rodent orthotopic models have been developed to address issues in drug development as well as biological origins of malignant gliomas. Establishing clinically relevant animal models of glioblastoma multiforme (GBM) remains a challenge, and many commonly used cell line-based models do not recapitulate the invasive growth patterns of patient GBMs. A novel orthotopic rat model of glioblastoma-the most malignant glioma in human-was recently developed, showing some stem-cell properties. The model is based on xenotransplantation of biopsy spheroids from human tumor tissue, into the brain of immunodeficient rats, where they initiated the growth of primary in most cases invasive and angiogenesis-independent glioblastomas. After serial passaging of tumors via spheroids in the subsequent generation of animals, the phenotype of the tumor changed. The most dramatic change was observed in approximately 1/3 of initially invasive tumors that changed into highly angiogenic and very aggressive. Some tumors though, remained invasive even after serial passages, while some were angiogenic from the start. The model thus provides combinations of angiogenic and invasive phenotypes and represents a good alternative to *in vitro* propagated cell lines for dissecting mechanisms of brain tumor progression. *In vivo* passaging of patient GBM biopsies produced tumors representative of the patient tumors, with high take rates and a reproducible disease course. The model has also been adapted to eGFP expressing immunodeficient mouse in which fluorescently marked tumors can be established *in vivo*.

Key words: malignant brain tumors; glioblastoma; nude rat; e-GFP NOD/SCID mice; xenograft; orthotopic transplantation; biopsy spheroids; translational medicine; molecular neuro-oncology

Introduction

Gliomas

Gliomas are brain tumors with some characteristics of brain support tissue – the glia. Malignant gliomas (anaplastic astrocytoma and glioblastoma multiforme–grade III and IV astrocytomas on WHO scale respectively) are predominant malignant brain tumors in human and have a poor prognosis with an average patient survival under current treatment regimens ranging between 12-14 months. Tumors are characterized by rapid cell growth, extensive neovascularization and diffuse cellular infiltration of normal brain structures (1). They have a relatively low overall incidence, but the fact that in spite of major progress in neurosurgery and oncology (including chemo-, radio- and, more recently biological-therapy) there hasn't been a major progress in malignant gliomas survival over several decades (2-7), urges the scientists to search for novel diagnostic tools, anti-tumor drug targets and therapies.

Received: 25 October 2010
Accepted for publication: 17 November 2010

Animal Tumor Models

In vivo animal modeling provides essential tumor-host interactions and is a more accurate way of mode-

ling human cancer than *in vitro*. We subdivide rodent models into xenograft tumor models – addressed in particular in this review - and models of spontaneous tumor formation in genetically engineered rodents. These models help us address important issues in drug development: toxicity and *in vivo* antitumor effectiveness (8, 9) (where the models are a matter of highly standardized procedures) as well as other basic phenomena in tumor origin and function such as angiogenesis, invasion and many others (3, 4, 10, 11) in the field of cancer research. *In vivo* modeling of drug efficacy is a gold standard required by a majority of pharmaceutical companies (12).

Xenograft tumors are initiated by implantation or injection of primary tumor cells or cell lines s.c. (under the skin) or orthotopically (into native tumor site) of syngeneic (genetically identical or closely related, so as to allow tissue transplant; immunologically compatible.), xenogeneic (derived or obtained from an organism of a different species, as a tissue graft), allogeneic (genetically different although belonging to or obtained from the same species, as in tissue grafts) or, immunosuppressed, immunodeficient, or newborn immunonaive animals (8, 9, 13, 14).

Rodent glioblastoma (GBM) models have been used for over 40 years but the extent to which they recapitulate the characteristics encountered in human GBMs remains controversial (8, 9). The advantages of xenograft glioma models are their highly efficient gliomagenesis, reproducible growth rates, and an accurate knowledge of the location of the tumor (6, 15, 23). However, in xenograft models stepwise genetic changes thought to occur during tumor progression are often missing; injected or implanted cells or cell lines have often been altered by culture or isolation conditions and lack their native tumor stroma. Murine models (e.g. (18) of GBM appear to recapitulate several of the human GBM histopathological features and, considering their reproducibility and availability, they constitute a valuable *in vivo* system for preclinical studies (8). On the other hand, many xenograft tumors lack histologicaly accurate vascularization, and rarely recapitulate tumor-of-origin phenotype (17). Immunodeficient rodents do not show antitumor immune effects and can produce false positives during drug trials (9). Syngeneic murine models, such as GL26 mouse glioma cells in C57BL6 mice (18) and CNS1 rat glioma cells in Lewis rats (19, 20) are non-immunogenic. Thus, syngeneic glioma models are excellent for studying the response of brain tumors to immunotherapy (19, 20). Establishing clinically relevant animal models of gliomas that would fully reflect the situation in human malignant glioma remains a challenge.

Two novel rat and mouse models to study glioblastoma

An orthotopic rat xenograft model of glioblastoma was developed (21) to address, among others, the tumor-host interaction issues leading to tumor circumscription, histologically accurate vascularization and to recapitulate accurately the tumor-of-origin phenotype. The model is based on a serial xenotransplantation of glioma biopsy spheroids, generated from glioma tissue (22) into the brain of nude rats. There the glioma spheroids induce the growth of primary, in most cases very invasive glioblastomas in 4-6 months time. These tumors co-opted the host vasculature and presented as an aggressive disease without signs of angiogenesis. The malignant cells expressed neural stem cell markers and showed a migratory behavior similar to normal human neural stem cells. When the rats became ill, they were sacrificed and spheroids were generated from their (human) tumors and implanted in the next generation of nude rats. This way, the tumors were passed in a total of 4-6 consecutive generations of nude rats (Figure 1). The most dramatic change was observed in approximately 1/3 of initially invasive tumors that changed into highly angiogenic and very aggressive, less invasive (more circumscribed), but which grew much faster due to good blood supply, killing a rat in 2-3 months. This switch to angiogenic phenotype was characterized by a reduction in stem cells markers (21, 23). Some tumors though, remained invasive even after serial passages, while some were angiogenic from the start. At the level of gene expression and immunoblotting proinvasive genes were up-regulated and angiogenesis signaling genes were down-regulated in invasive tumors. In contrast, proinvasive genes were down-regulated in the angiogenesis-dependent tumors derived from the invasive tumors (21). Uncoupling of invasion and angiogenesis, represented by the stem-like cancer cells and the cells derived from them respectively, points at two different mechanisms that drive tumor progression. Although the mechanism behind the phenotypic shift is not fully understood, HIF-1 expression seems to be triggered by hypoxia, because it was not constitutively expressed by high-generation tumor spheroids cultured under normoxic conditions (21) .

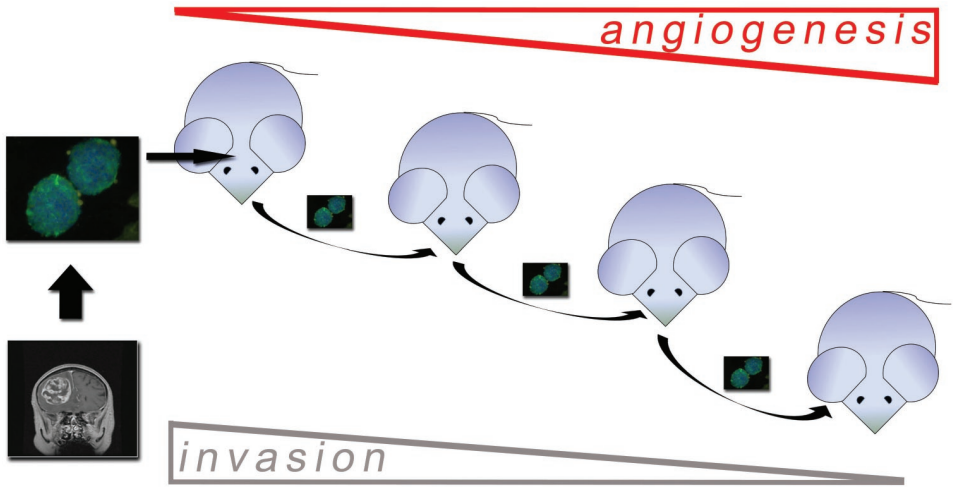


Figure 1: The orthotopic rat glioblastoma model

Thus, by serial passaging, this model uncouples and recapitulates for the first time the two major phenotype characteristics of human glioblastoma which makes it one of the best available glioblastoma models and opens the door to multiple applications in basic, translational and pre-clinical research. The major difference and - possibly – the advantage of this model over other described rodent models of glioblastoma (8, 9) arises from the fact that in the biopsy spheroids, which are structures of heterogenic cellular population, at least part of original glioma microenvironment is conserved, a part essential in inducing the tumor upon transplantation. This microenvironment is absent in xenografts generated from immortalized glioma cell lines (8, 9), but may be conserved to some extent in primary glioma cell lines derived from glioma patients and in glioma tissue grafts. The phenotypes, derived from the same tumor sample, may develop through a selection process where most cells in the biopsy specimens die following implantation in the rat brain. The cells that survive show stem cell-like properties, and are able to adapt to the new microenvironment where they divide and produce new tumor clones that show rapid growth and angiogenesis (21, 24).

The concept of the Rodent Glioblastoma model

Samples of glioblastoma, resected from the patients are finely minced and grown in cell culture medium. There the cells organize in tumor biopsy spheroids of about 300μm in size. Spheroids are orthotopically implanted into the brain of immunodeficient rodents. There, in most cases, they induce the growth of primary glioblastomas of invasive nature, but non-angiogenic in 4-6 months. With serial passaging in the subsequent generations of rodents via spheroids, the tumor phenotype will change in 1/3 of cases to fully angiogenic in the last generation xenograft with an onset of tumor in 2-3 months.

eGFP NOD/Scid mouse model

This glioblastoma model was recently adapted to a NOD/Scid mouse expressing enhanced green fluorescent protein (eGFP) (Figure 2). In this model human and mouse tumors marked with red fluorescent protein can be established *in vivo*, both at subcutaneous and orthotopic locations (25). Using modern microscopy techniques, the intricate co localization of tumor and host cells *in situ* can be visualized in detail. Technology of fluorescence-activated cell sorting (FACS), allows for complete and reliable separation of the host cells from the tumor cells, thus providing a system for detailed cellular and molecular analysis of tumor-host cell interactions. The fact that tumor and host cells can be reliably identified also allows for detection of double-positive cells, possibly arising from cell fusion events or horizontal gene transfer (two possible origins of tumor initiating – tumor stem cells; (26)). Similarly, the model can be applied for the detection of circulating metastatic cells and for detailed studies on the vascular compartments within tumors, including vasculogenic mimicry (25). Thus, the model should provide significant insight into how tumor cells communicate with their microenvironment.

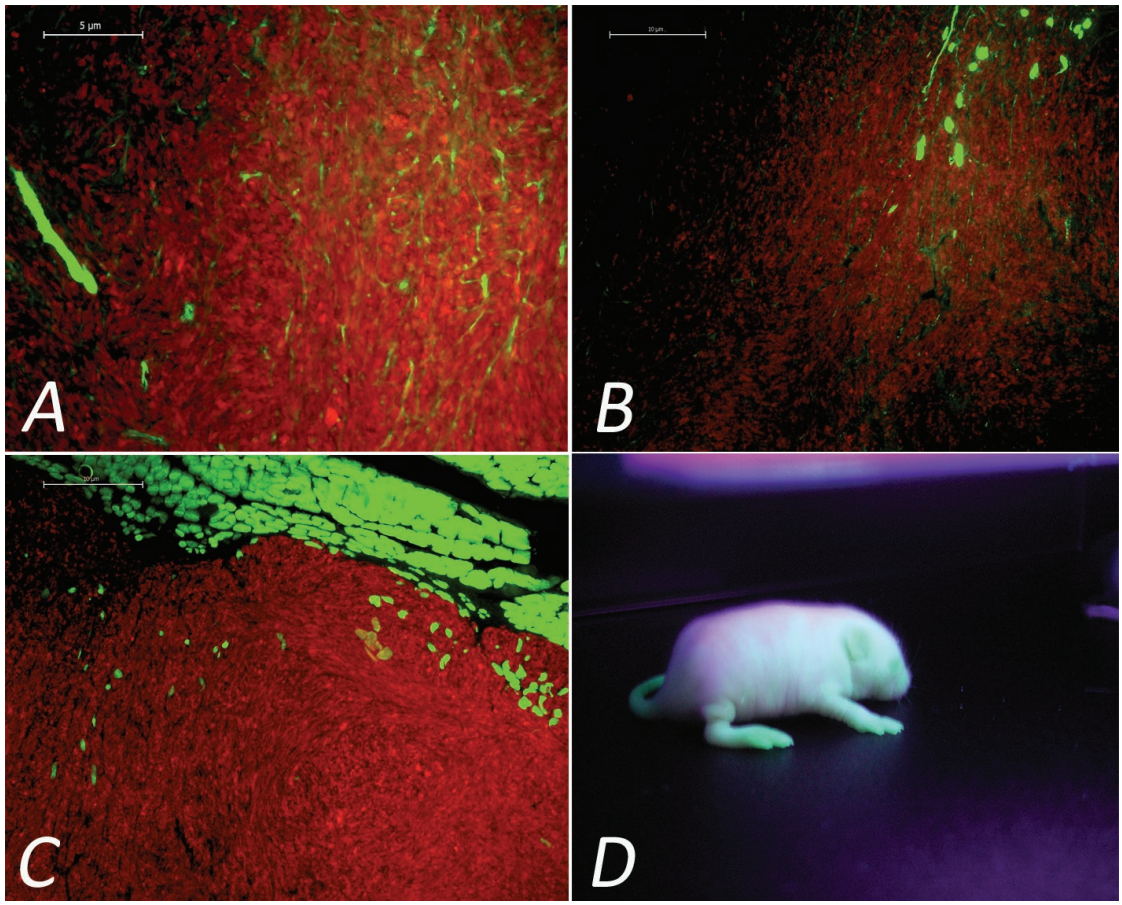


Figure 2: Tumor host interaction studies in an eGFP NOD/Scid mouse model

U87 dsRed expressing human glioblastoma cells were implanted in the flank of eGFP NOD/Scid mouse. (A-C) Endogenous fluorescence on a frozen histological section from a U87 subcutaneous tumor showing eGFP-expressing host cells within the tumor bed (direct eGFP and dsRed fluorescence). Scale bars = 5 µm (A); 10 µm (B, C). (D) a newborn eGFP NOD/Scid mouse glowing in green fluorescence under the UV light

Applications of the rodent glioblastoma model

Since its establishment, the rat model of glioblastoma was characterized in detail on several levels and used in multiple assays ranging from basic research to drug testing.

MR spectroscopy

In this study the metabolic properties of the two experimental phenotypes were analyzed. The early generation (EG) showed an intact blood–brain barrier and normal vascular morphology. In contrast, the high generation (HG) exhibited leaky vessels and necrosis. The rats with HG tumor had raised concentrations of choline and myo-inositol, and decreased concentrations of glutamate and N-acetylaspartate. In the LG tumor group, similar changes in metabolic concentrations were detected, although the alterations were more pronounced. The LG tumors also had higher concentrations of choline, taurine, and lactate. Results of this study showed that metabolic profiles could be used to distinguish between two glioblastoma phenotypes. More pronounced anaerobic metabolism was present in the LG stem-cell-like tumors, suggesting a more malignant phenotype (27).

Table 1: Applications of the rodent glioblastoma model

Applications		Model	Reference	Comments
Metabolite analysis	Determination of metabolic properties of the xenografts by Magnetic Resonance Spectroscopy (MRS)	Nude rat glioma xenograft	Thorsen F., et al., 2008 (27)	Metabolic profiles produced by MRS could be used to distinguish between two distinct glioblastoma phenotypes
Tumor initiation, take and reproducibility	Othotopic, stereotactic glioblastoma xenograft model construction and characterization	Nude rat glioma xenograft	Sakariassen PO et al., 2006 (21)	Separation of early /invasive tumor phenotypes and late/angiogenic tumors by serial passaging in nude rats
	Tumorigenesis; neuropathological and radiological features of rat glioblastoma xenograft model	Nude rat glioma xenograft	Wang J et al., 2009 (23)	In vivo passaging of patient GBM biopsies produced tumors representative of the patient tumors, with high take rates and a reproducible disease course.
	Tumor-host interaction studies	eGFP expressing NOD/SCID mouse	Niclou SP et al., 2008 (25)	Fluorescence-based intricate co localization of tumor and host cells *in situ*.
Anti-tumor drug testing and glioma treatment studies	Radio surgery	glioblastoma spheroids, nude rat glioma xenograft	Thorsen FA et al, 2007 (30)	Radio surgery of malignant gliomas might be effective in controlling tumor progression in selected glioblastoma patients.
	The response of the two phenotypes to doxorubicin	Nude rat glioma xenograft	Johannessen T-CA et al., 2009, (24)	Highly invasive tumors shown to be more chemo resistant than angiogenic tumors derived from the same patients.
	The effect of hyperoxic treatment on BT4C rat glioma xenografts	BT4C rat glioma xenografts	Stuhr LEB et al., 2007 (31)	Increased pO2–levels in experimental gliomas, using normobaric and moderate hyperbaric oxygen therapy, caused a significant reduction in tumor growth, a process characterized by enhanced cell death, reduced vascular density and changes
Gene Therapy	Adenoviral vector (AAV) transduction	Glioma cell lines, glioblastoma spheroids, nude rat glioma xenograft	Thorsen FA et al., 2006 (32)	AAV4 and AAV5 serotypes may be used to transduce biologically diverse glioma cell lines. They also penetrate and transduce solid human tumor tissue derived from patient biopsies.
	Lentiviral vector transduction	human embryonic kidney cell line 293T, TE671 cell line, nude rat glioma xenograft	Huszthy PC et al., 2009 (33)	Lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) and vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral vectors efficiently transduced human glioblastoma cells and cancer stem-like cells. Pseudotyped gamma retroviral vectors, similar to those evaluated for clinical therapy of glioblastoma, showed inefficient gene transfer.
	Oncolytic HSV-1 based vector G207	Nude rat glioma xenograft	Huszthy PC et al., 2010, (34)	Favorable cellular responses to G207 treatment seen from a clinical viewpoint, such as reduced tumor cell proliferation, more frequent events of tumor cell death and a strongly attenuated tumor vascular compartment.
Biomarker research	The glioma-associated gangliosides 3k-isoLM1, GD3 and GM2	Nude rat glioma xenograft	Hedberg KM et al, 2001, (35)	Different biological roles for individual gangliosides; antibodies or ligands directed against GD3 and 3k-isoLM1 might be complementary when applied in the treatment of human glioblastomas.
	Expression of extracellular matrix components in a highly infiltrative glioma model	Glioma cell lines, nude rat glioma xenograft	Mahesparan R et al., 2003, (16)	Possible biological function of tenascin, vitronectin, laminin, fibronectin and collagen type IV in highly invasive malignant tumors of glial origin.
	PDI protein expression in invasive phenotype tumors	Nude rat glioma xenograft	Goplen D et al., 2006 (36)	PDI was shown to be expressed on migrating and invading glioma cells.
	Tumor initiating cell markers	Nude rat glioma xenograft	Wang J et al., 2007(29)	Cd133- glioma cells are tumorigenic and can produce cd133+ tumors, CD133 expression coincides with the onset of angiogenesis and a shorter survival
	Global membrane proteomics	Nude rat glioma xenograft	Rajcevic U et al., 2009 (37)	Known and novel candidate proteins were identified that characterize the switch from a non-angiogenic to a highly angiogenic phenotype. Enhanced intercellular cross-talk and metabolic activity adopted by tumor cells in the angiogenic compared with the non-angiogenic phenotype.
	Neural cell adhesion molecule (NCAM) as a glioma marker for the biological aggressiveness	Nude rat glioma xenograft	Duenisch P et al., 2010, (38)	The expression of NCAM-140 inversely correlated with the WHO grade of human gliomas

Tumor initiation, take and reproducibility

The tumor take rates for xenografted GBM biopsies were 96% and close to 100% at subsequent passages *in vivo*. Only one of four lower grade tumors engrafted. MRI typically revealed changes related to tumor growth, several months prior to the onset of symptoms (23).

In another study, CD133 expression was analyzed at various passages. CD133 is a cell surface marker identified as a putative marker of brain tumor-initiating cells (28). During passaging, the tumors gradually displayed more contrast enhancement, increased angiogenesis, shorter survival and increased CD133 expression. CD133 negative cells derived from 6 different patients were tumorogenic when implanted into the rat brains. For 3 of these patients, analysis showed that the resulting tumors contained CD133 positive cells. In this assay, the authors showed that CD133 negative glioma cells were tumorogenic in nude rats, and that CD133 positive cells can be obtained from these tumors. Upon passaging of the tumors *in vivo*, CD133 expression is up regulated, coinciding with the onset of angiogenesis and a shorter survival. Authors also suggested that CD133 may not be essential in tumor initiation process (29).

Anti-tumor drug testing and glioma treatment studies

In a study focused on the radiobiological effects of the Gamma knife (Gamma Knife is currently used to boost treatment of malignant gliomas) the growth and invasiveness of human glioblastoma spheroids xenografted into nude rat brains were assessed after radio surgery. A dose-dependent inhibition of tumor growth and invasion, as well as a dose-dependent increase in animal survival was observed. The results indicated that radio surgery of malignant gliomas might be effective in controlling tumor progression in selected glioblastoma patients (30).

In a study aimed at investigating how the two phenotypes responded *in vitro* to doxorubicin, a clinically potent cytotoxic drug for solid tumors, highly invasive tumors shown to be more chemo resistant than angiogenic tumors derived from the same patients. It was suggested that treatment resistance in glioblastomas could be related to PI3K/AKT activity in stem-like tumor cells, and that targeted interference with the PI3K/AKT pathway might differentiate and sensitize this subpopulation to chemotherapy (24).

Another study described the biological effects of hyperoxic treatment on BT4C rat glioma xenografts *in vivo* with special reference to tumor growth, angiogenesis, apoptosis, general morphology and gene expression parameters. Increased pO2–levels in experimental gliomas, using normobaric and moderate hyperbaric oxygen therapy, caused a significant reduction in tumor growth. This process was characterized by enhanced cell death, reduced vascular density and changes in gene expression corresponding to these effects (31).

Gene Therapy

In one of the initial studies on the delivery vehicles for gene transfer strategies directed at the central nervous system (CNS), muscle and liver performed on the featuring model the transduction efficacy of AAV serotypes 4 and 5 were compared to AAV2, both *in vitro* and in intracranial GBM xenografts . While all three AAV serotypes were able to transduce the glioma cell lines when added individually or when they were administered in concert, AAV2 transduced the glioma cells most effectively compared to AAV4 or AAV5. Upon infecting glioblastoma spheroids *in vitro*, all three AAV serotypes efficiently transduced cells located at the surface as well as within deeper layers of the spheroids. In addition, both AAV4 and AAV5 were able to transduce human glioblastoma xenografts implanted intracranially. Authors suggested that beside AAV2 serotype, AAV4 and AAV5 serotypes may also be used to transduce biologically diverse glioma cell lines and may be used in developing treatment vehicles for human malignant gliomas (32).

The rat xenograft model was used to analyze the transduction pattern and therapeutic efficacy of lentiviral pseudotyped vectors. Both, lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) and vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral vectors efficiently transduced human glioblastoma cells and cancer stem-like cells. In contrast, pseudotyped gamma retroviral vectors, similar to those evaluated for clinical therapy of glioblastoma, showed inefficient gene transfer. In a therapeutic approach using the suicide gene herpes simplex virus thymidine kinase (HSV-1-tk) fused to eGFP, both lentiviral vectors mediated a complete remission. In all recurrent tumors, surviving eGFP-positive tumor cells were found, advocating prodrug application for several cycles to even enhance and prolong the therapeutic effect. The inefficient gene delivery by gamma retroviral vectors is in line with the results obtained in clinical therapy for GBM and thus con-

firms the high reproducibility of the invasive glioma animal model for translational research (33).

In another assay the cellular effects of the oncolytic HSV-1 based vector, G207, on the tumor microenvironment were evaluated. The xenografted tumors were quantitatively evaluated 10-30 days after G207 injection for virus-induced changes in proliferation, apoptosis and vascularity. Vector spread and the infiltration pattern of CD68-positive inflammatory cells were assessed. Proliferation indices were lower, whereas apoptotic counts were elevated in plaques as compared with that in non-infected areas of the same lesions, as well as in corresponding control xenografts. A decline in the number of blood vessels was noticed in the plaques and the vascular area fractions were reduced. CD68-positive inflammatory cells accumulated in the plaques. The study highlighted the favorable cellular responses to G207 treatment seen from a clinical viewpoint, such as reduced tumor cell proliferation, more frequent events of tumor cell death and a strongly attenuated tumor vascular compartment (34).

Biomarker research

A substantial number of biomarker research studies were thus far performed on the model. Initially roles for glioma-associated gangliosides (3k-isoLM1, GD3 and GM2) were assessed in the model, pointing out that different biological roles for individual gangliosides may exist and that the antibodies or ligands directed against GD3 and 3k-isoLM1 might be complementary when applied in the treatment of human glioblastomas (35).

In a different study expression of extracellular matrix (ECM) components in this highly infiltrative *in vivo* glioma model was analyzed. The cellular origin for several of these ECM components was identified using human-specific monoclonal antibodies and polyclonal antibodies detecting epitopes from both species (human and rat). Observed staining patterns clarified the cellular origin and indicated the possible biological function of tenascin, vitronectin, laminin, fibronectin and collagen type IV in these highly invasive malignant tumors of glial origin (16).

In the initial proteomic assays on biomarkers on the featuring model the researchers showed that PDI, one of the most prominently up-regulated proteins in invasive tumors is strongly expressed on invasive glioma cells, in both xenografts and at the invasive front of human glioblastomas. Using *in vitro* assays, PDI was shown to be expressed on migrating glioma cells. Functional significance of PDI in cell migration and invasion was tested *in vitro*, showing an important role of PDI in glioma cell invasion (36).

In a global proteomics comparison of the two xenograft phenotypes we were able to identify several thousand proteins in membrane-enriched fractions of which 1460 were extracted as quantifiable proteins (isoform- and species specific and present in more than one sample). Known and novel candidate proteins were identified that characterize the switch from a non-angiogenic to a highly angiogenic phenotype. The data pointed to enhanced intercellular cross-talk and metabolic activity adopted by tumor cells in the angiogenic compared with the non-angiogenic phenotype. The identified proteins could be further exploited as biomarkers or therapeutic targets for malignant gliomas (37).

In a different assay the relevance of neural cell adhesion molecule (NCAM) as a glioma tissue marker for the biological aggressiveness of these tumors was assessed. The expression of NCAM-140 inversely correlated with the WHO grade of human gliomas. The lost expression of NCAM-140 in human glioblastomas and in brain metastases enabled the investigation of the brain– tumor interface and the definition of glioblastoma invasion patterns and showed that brain metastases are more invasive than ever thought (38).

Conclusions

In vivo passaging of patient GBM biopsies in rats or mice produced tumors representative of the patient tumors, with high take rates and a reproducible disease course. The main advantage of the model provides combinations of angiogenic and invasive phenotypes and represents a good alternative to *in vitro* propagated cell lines for dissecting mechanisms of brain tumor progression (Wang J, BMC Cancer 2009) as well as to other *in vivo* orthotopic rodent GBM models. Thus far, the model has been extensively used in a variety of applications, ranging from basic research of cancer biology and biomarkers including genomics, transcriptomics, proteomics and metabolomics, through translational research of therapeutics and therapeutic modalities to preclinical research of drugs approved for use in other types of cancer. The results of this research provided important novel insights into the mechanisms of tumor initiation, promotion and progression

through transcriptomic markers, through novel protein biomarkers, validated on clinical and pre-clinical material. Results also indicated the metabolic changes linked to phenotype switch in the model. A possibility of separating the tumor-host compartments in the model based on cellular, fluorescence based- or biochemical, protein sequence-based level enables an unprecedented potential in tumor-host interaction studies, the complex of events crucial for the understanding of tumor initiation and function. The research of novel therapeutics and therapeutic modalities has shown a great potential of this model as it recapitulates the main features of the GBM, for *in vivo* studies including radio-, chemo- and gene therapies.

Acknowledgements

Dr Simone P. Niclou, NorLux Neuro-Oncology Laboratory, CRP-Santé, Luxembourg is kindly acknowledged for the critical review of the manuscript and for providing photos of the animal model. The work was funded by Fonds National de la Recherche (FNR), Luxembourg (contract FNR-AFR # PDR-08-007) to U.R. and by Slovene Research Agency (Program Grant # P1—0245).

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumors of the central nervous system. Acta Neuropathol 2007;114:97-109.

2. Castro MG, Cowen R, Williamson IK, et al. Current and future strategies for the treatment of malignant brain tumors. Pharmacol Ther 2003;98:71-108.

3. Chiocca EA. Oncolytic viruses. Nat Rev Cancer 2002;2:938-50.

4. Curtin JF, King GD, Candolfi M, et al. Combining cytotoxic and immune-mediated gene therapy to treat brain tumors. Curr Top Med Chem 2005;5:1151-70.

5. Gomez-Manzano C, Yung WK, Alemany R, Fueyo J. Genetically modified adenoviruses against gliomas: from bench to bedside. Neurology 2004;63:418-26.

6. King GD, Curtin JF, Candolfi M, Kroeger K, Lowenstein PR, Castro MG. Gene therapy and targeted toxins for glioma. Curr Gene Ther 2005;5:535-57.

7. Prados MD. Future directions in the treatment of malignant gliomas with temozolomide. Semin Oncol 2000;27:41-6.

8. Candolfi M, Curtin JF, Nichols WS, et al. Intracranial glioblastoma models in preclinical neuro-oncology: neuropathological characterization and tumor progression. J Neurooncol 2007;85:133-48.

9. Fomchenko EI, Holland EC. Mouse models of brain tumors and their applications in preclinical trials. Clin Cancer Res 2006;12:5288-97.

10. Fecci PE, Mitchell DA, Archer GE, et al. The history, evolution, and clinical use of dendritic cell-based immunization strategies in the therapy of brain tumors. J Neurooncol 2003;64:161-76.

11. Rainov NG, Ren H. Gene therapy for human malignant brain tumors. Cancer J 2003;9:180-8.

12. Seller WR. Cancer drug development in the modern era. In: EC H, ed. Mouse models of human cancers. Hoboken (New Jersey): John Wiley and Sons; 2004.

13. Lassmann S, Opitz OG. The new look of colorectal cancer stem cells. Gastroenterology 2008;134:1262-4.

14. Shapiro WR, Basler GA, Chernik NL, Posner JB. Human brain tumor transplantation into nude mice. J Natl Cancer Inst 1979;62:447-53.

15. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumor progression. Nat Rev Cancer 2005;5:744-9.

16. Mahesparan R, Read TA, Lund-Johansen M, Skaftnesmo KO, Bjerkvig R, Engebraaten O. Expression of extracellular matrix components in a highly infiltrative in vivo glioma model. Acta Neuropathol 2003;105:49-57.

17. Finkelstein SD, Black P, Nowak TP, Hand CM, Christensen S, Finch PW. Histological characteristics and expression of acidic and basic fibroblast growth factor genes in intracerebral xenogeneic transplants of human glioma cells. Neurosurgery 1994;34:136-43.

18. Albright L, Madigan JC, Gaston MR, Houchens DP. Therapy in an intracerebral murine glioma model, using Bacillus Calmette-Guerin, neuraminidase-treated tumor cells, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Cancer Res 1975;35:658-65.

19. Ali S, Curtin JF, Zirger JM, et al. Inflammatory and anti-glioma effects of an adenovirus expressing human soluble Fms-like tyrosine kinase 3 ligand (hsFlt3L): treatment with hsFlt3L inhibits intracranial glioma progression. Mol Ther 2004;10:1071-84.

20. Ali S, King GD, Curtin JF, et al. Combined immunostimulation and conditional cytotoxic gene therapy provide long-term survival in a large glioma model. Cancer Res 2005;65:7194-204.

21. Sakariassen PO, Prestegarden L, Wang J, et al. Angiogenesis-independent tumor growth mediated by stem-like cancer cells. Proc Natl Acad Sci U S A 2006;103:16466-71.

22. Bjerkvig R, Tonnesen A, Laerum OD, Backlund EO. Multicellular tumor spheroids from human gliomas maintained in organ culture. J Neurosurg 1990;72:463-75.

23. Wang J, Miletic H, Sakariassen PO, et al. A reproducible brain tumor model established from human glioblastoma biopsies. BMC Cancer 2009;9:465.

24. Johannessen TC, Wang J, Skaftnesmo KO, et al. Highly infiltrative brain tumors show reduced chemosensitivity associated with a stem cell-like phenotype. Neuropathol Appl Neurobiol 2009;35:380-93.

25. Niclou SP, Danzeisen C, Eikesdal HP, et al. A novel eGFP-expressing immunodeficient mouse model to study tumor-host interactions. Faseb J 2008;22:3120-8.

26. Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. Opinion: the origin of the cancer stem cell: current controversies and new insights. Nat Rev Cancer 2005;5:899-904.

27. Thorsen F, Jirak D, Wang J, et al. Two distinct tumor phenotypes isolated from glioblastomas show different MRS characteristics. NMR Biomed 2008;21:830-8.

28. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumor initiating cells. Nature 2004;432:396-401.

29. Wang J, Sakariassen PO, Tsinkalovsky O, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer 2008;122:761-8.

30. Thorsen F, Enger PO, Wang J, Bjerkvig R, Pedersen PH. Human glioblastoma biopsy spheroids xenografted into the nude rat brain show growth inhibition after stereotactic radio surgery. J Neurooncol 2007;82:1-10.

31. Stuhr LE, Raa A, Oyan AM, et al. Hyperoxia retards growth and induces apoptosis, changes in vascular density and gene expression in transplanted gliomas in nude rats. J Neurooncol 2007;85:191-202.

32. Thorsen F, Afione S, Huszthy PC, et al. Adeno-associated virus (AAV) serotypes 2, 4 and 5 display similar transduction profiles and penetrate solid tumor tissue in models of human glioma. J Gene Med 2006;8:1131-40.

33. Huszthy PC, Giroglou T, Tsinkalovsky O, et al. Remission of invasive, cancer stem-like glioblastoma xenografts using lentiviral vector-mediated suicide gene therapy. PLoS One 2009;4:e6314.

34. Huszthy PC, Immervoll H, Wang J, et al. Cellular effects of oncolytic viral therapy on the glioblastoma microenvironment. Gene Ther 2010;17:202-16.

35. Hedberg KM, Mahesparan R, Read TA, et al. The glioma-associated gangliosides 3'-isoLM1, GD3 and GM2 show selective area expression in human glioblastoma xenografts in nude rat brains. Neuropathol Appl Neurobiol 2001;27:451-64.

36. Goplen D, Wang J, Enger PO, et al. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. Cancer Res 2006;66:9895-902.

37. Rajcevic U, Petersen K, Knol JC, et al. iTRAQ based proteomic profiling reveals increased metabolic activity and cellular crosstalk in angiogenic compared to invasive Glioblastoma phenotype. Mol Cell Proteomics 2009;8:2595-612.

38. Duenisch P, Reichart R, Mueller U, et al. Neural cell adhesion molecule isoform 140 declines with rise of WHO grade in human gliomas and serves as indicator for the invasion zone of multiform glioblastomas and brain metastases. J Cancer Res Clin Oncol 2011;137:399-414.

GLODAVSKI MOŽGANSKI ORTOTOPIČNI MODEL ZA ŠTUDIJ ČLOVEŠKEGA MALIGNEGA GLIOMA

U. Rajčević

Povzetek: Maligni gliomi so neozdravljivi možganski tumorji. Se relativno redko pojavljajo, vendar ne glede na izboljšave pri terapiji večjega napredka pri preživelosti bolnikov ni bilo že več desetletij. Zaradi tega je pomembno raziskovanje novih diagnostičnih orodij, ugotavljanje novih tarč v tumorskem tkivu in razvijanje novih načinov zdravljenja. Zdi se, da naša nezmožnost uspešno pozdraviti raka na možganih izvira iz nepoznavanja bioloških in biokemičnih lastnosti tumorjev. Z leti je nastalo več glodavskih ortotopičnih modelov, ki so lahko uporabni pri razvoju novih zdravil in pri proučevanju bioloških osnov malignih gliomov. Razvoj klinično pomembnih živalskih modelov za multiformni glioblastom (GBM) ostaja izziv in številni, pogosto uporabljeni modeli na osnovi celičnih linij, ne odražajo vzorcev invazivne rasti GBM pri bolnikih. Nedavno je bil razvit nov ortotopični podganji model glioblastoma – najbolj maligne oblike glioma pri človeku, ki kaže nekatere značilnosti, podobne matičnim celicam. Model je nastal na osnovi medvrstne presaditve biopsijskih sferoidov iz vzorcev človeških tumorjev v možgane podgan z oslabljenim imunskim sistemom, kjer so sferoidi povzročili rast primarnih in večinoma invazivnih, od angiogeneze neodvisnih glioblastomov. Po zaporednem prenosu teh tumorjev preko sferoidov v naslednje generacije živali se je fenotip tumorja spremenil. Najbolj dramatična sprememba je bila ugotovljena pri približno tretjini sprva invazivnih tumorjev, ki so se spremenili v visoko angiogene in zelo agresivne. Nekaj tumorjev je ostalo invazivnih kljub zaporednemu prenosu v naslednje generacije podgan, medtem ko so bili nekateri angiogeni že od začetka. Model torej prinaša kombinacijo angiogenih in invazivnih fenotipov in predstavlja dobro alternativo celičnim linijam, gojenim v pogojih *in vitro*, za ugotavljanje mehanizmov napredovanja možganskih tumorjev. Zaporedni prenos bolnikovih biopsij GBM *in vivo* je povzročil nastanek tumorjev, podobnih pacientovim, z visoko ravnijo tumorigeneze in ponovljivim potekom bolezni. Model je bil prilagojen tudi mišim z oslabljenim imunskim odgovorom, ki izražajo zeleno fluorescenčno beljakovino in kjer lahko vzpodbudimo nastanek fluorescenčno označenih tumorjev pri živih živalih.

Ključne besede: maligni možganski tumorji; glioblastom, gole podgane; miši e-GFP NOD/SCID; ksenograft; ortotopična transplantacija; biopsijski sferoidis; translacijska medicina; molekularna nevroonkologija

Slov Vet Res 2011; 48 (1): 15-26
UDC 636.4.083:631.147:637.51

Original Scientific Article

GROWTH, CARCASS AND MEAT QUALITY TRAITS OF PIGS RAISED UNDER ORGANIC OR CONVENTIONAL REARING SYSTEMS USING COMMERCIALLY AVAILABLE FEED MIXTURES

Maja Prevolnik[1, 2], Marko Ocepek[1], Marjeta Čandek-Potokar[1, 2], Martina Bavec[1], Dejan Škorjanc[1]*

[1]Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia; [2]Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: dejan.skorjanc@uni-mb.si

Summary: The objective of the study was to evaluate performance from birth until slaughter, as well as final carcass and meat quality, of pigs raised either conventionally (n=32) or respecting organic standards (n=35) using commercially available organic feed mixtures. Lower (22%) feed intake from weaning until week 11 was noted for organic pigs. As a consequence, organic pigs had lower growth rate persisting until week 22. In the last phase (weeks 22 to 26) when food intake was limited, growth rate declined in conventional, and increased in organic, pigs. All pigs were slaughtered at the usual commercial age (26 weeks). Due to the slower growth, organic pigs had lower carcass weight, dressing %, smaller *longissimus dorsi* (LD) muscle, and lighter hams, while no differences were observed in fat tissue measurements and carcass leanness (the exception being the area of fat over LD). Analysis for the same slaughter weight indicated that organic pigs would have fatter carcasses as conventional pigs if slaughtered at the same weight. With regard to meat quality, a higher ultimate pH and intramuscular fat content were observed for organic pigs. The results of the present study indicate possible problems (lower feed intake, growth retardation) associated with the use of commercially available organic diets for piglets. On the other hand such diets can increase intramuscular fat content, which is interesting in terms of improved meat quality.

Key words: organic farming; growth; carcass quality; meat quality; stress markers; pig

Introduction

In most European countries, organic pig production has so far played a minor role and the proportion of organically produced pigs is very low (less than 1% (1,2). However, there is a general interest in the European Union (EU) in organic production systems. Consumers often perceive organic food to be healthier, safer, and more palatable and nutritious, owing to the recognition that organic food is wholesome, environmental friendly, traditional and sustainable (3).

Received: 9 November 2010
Accepted for publication: 31 January 2011

Owing to the specific standards and requirements, the costs of organic production could be up to 30% higher than those of conventional rearing (4). The legislation associated with organic pig production is aimed at animal welfare, including the provision of environmental conditions that allow livestock to perform their normal behaviour patterns. The animals must be free from hunger and thirst, thermal and physical discomfort, and they must be protected from injuries, diseases, fear and stress. The legislation (5) provides requirements that include specifications for housing conditions, animal nutrition, and animal breeding, as well as animal care, disease prevention and veterinary treatment.

Many studies have been carried out to determine if the consumer perceptions, that quality of organic food is better, are supported scientifically. Growth performance and the carcass and meat quality of pigs from organic and conventional production systems have been compared, and the published results show inconsistencies (1,2,6-11). Discrepancies in the published results can be related to housing, feed, breed or genotype, and their interactions, which are reflected in the health, welfare and productivity of pigs.

In view of the inconsistency of the research reports and to test local conditions, an experiment was conducted with the objective to evaluate growth performance and carcass and meat quality traits of pigs reared conventionally or respecting organic standards, but using commercially available organic feedstuffs. Contrary to the majority of the studies, where pigs were put to the test at 20–30 kg live weight, the objective of our experiment was to follow the growth performance of pigs from birth until slaughter and to evaluate the consequences for carcass and meat quality.

Materials and methods

Animals, housing and feed

The study was conducted at the Pig Research Centre of Faculty of Agriculture and Life Sciences, University of Maribor, Slovenia, and national legislation on animal protection (12) was respected. The experimental pigs were the progeny of LANDRACE×LARGE WHITE dams and LANDRACE×PIETRAIN sires (free of the *RYR1* gene). The pigs originated from eight sequential litters born within two months period. The piglets in the first four litters formed a group that was reared according to conventional practices of housing and feeding (n=35; 13 females and 19 castrates). Pigs from the other four litters formed a group of pigs (n=40; 15 females and 20 castrates) that were reared respecting the constraints of EU legislation on organic production (5). Surgical castration of the male pigs was performed at the age of three or four days. Owing to mortality, the final analysis comprised 67 pigs (n=32 conventional, and n=35 organic pigs).

Table 1: Available space in conventional and organic pigs according to rearing phase

	Available space	
Rearing phase	Conventional	Organic
Lactation (4 or 6 weeks for conventional or organic group, respectively)	Pen size 1.8×2.5m^2	Pen size 1.8×2.5m^2
Growing (weeks 4-11 or 6-11 for conventional or organic group, respectively)	Pen size 1.8×2.0m^2 0.26-0.36 m^2/piglet	Pen size 3.8×2.3m^2 0.62-0.67 m^2/piglet Straw bedding Outdoor area 2.9×2.3m^2
Finishing (weeks 11-26 for both groups)	Pen size 3.8×2.1m^2 0.66-0.72 m^2/piglet	Pen size 3.8×2.1m^2 1.33 -1.59 m^2/piglet Straw bedding Outdoor area 2.9×2.1m^2

The differences between organic and conventional rearing system are detailed below and in Table 1 and Table 2. Organic pigs had a larger rearing space, access to an outdoor area, straw bedding, longer lactation, and received a commercially available organic feed mixture. Pigs were weaned after 28 days (conventional) or 42 days (organic) of lactation. After weaning, pigs from the conventional group were allotted to three pens for the whole fattening period. Pigs from the organic group were housed in three pens until week 11 when they were further divided into seven pens in order to fulfil space requirements. The available space (Table 1) was in agreement with relevant legislation (5,12).

Pigs were fed with a commercially available feed mixture in pellets (Table 2). The same feeding strategy was used for all pigs, except that the organic pigs received feed mixture of organic origin. From the second week of lactation, in addition to their intake of milk, all the piglets were provided with additional feed mixture (in pellets). During the last four weeks of fattening, only 2.2 kg of feed mixture per pig was provided daily in order to limit fat deposition.

Table 2: Composition of experimental diets[A]

	Conventional			Organic	
Feed mixture	Prestarter[1]	Bek-1 farm[1]	Bek-2 farm[1]	Alpenkorn Ferkel[2]	Alpenkorn Schweine[2]
Rearing phase	Lactation & post-weaning 2-8 weeks	Growing 8-16 weeks	Finishing 16-26 weeks	Lactation & post-weaning 2-9 weeks	Growing & Finishing 9-26 weeks
Diet ingredients	maize 38%, barley 12%, soya meal 17%,milk powder 8%, wheat 8%, fish flour 5%, soya concentrate 2%, minerals, oils & fats, vitamins, Lys, aromatic substances, enzymes, organic acids	maize 35%, barley 22%, soya meal 18%, wheat 15%, sunflower meal 3%, fish meal 1%, lucerne 1%, minerals, oils and fats, vitamins, Lys, aromatic substances, enzymes	maize 34%, barley 23%, soya meal 15%, wheat 11%, sunflower meal 4%, lucerne 2%, minerals, oils and fats, vitamins, Lys, aromatic substances, enzymes	wheat 50%, wheat bran 11%, faba bean 10%, soybean 7%, triticale 6%, barley 5%, rape cake 3%, potato proteins 3%, molasses 2%	triticale 25%, wheat 15%, wheat bran 12%, barley 9%, maize 7%, sunflower cake 9%, rape cake 7%, soybean 5%, faba bean 3%, potato proteins 2.5%, molasses 2%
CP, g/kg	206	169	158	170	170
ME, MJ/kg	12.3	12.0	12.0	12.8	12.5
Lys, g/kg	12.5	10.0	7.0	8.0	8.0

[A]Conventional and organic feed mixtures were commercially available; CP - crude proteins; ME - metabolizable energy; Lys – lysine; [1]supplier Perutnina Ptuj d.d., PC Krmila, DE Proizvodnja krmil, Draženci, SI-2250 Ptuj; [2]supplier UNSER LAGERHAUS Warenhandels Ges.m.b.h., Mischfutterwerk, Südring 240, A-9020 Klagenfurt

The pigs were weighed individually one day after birth and at 4, 6, 11, 22 and 26 weeks of age. Daily gains were calculated using the data on body weight and age at weighing for the whole experimental period (0 to 26 weeks; life daily gain) and for the periods between sequential weight measurements. Feed intake was recorded per pen and divided by the number of pigs to give the daily intake per pig. Feed conversion efficiency was calculated per pen as the ratio between feed intake and live weight gain for each period from weaning until slaughter (week 4 or 6 to 26).

Transport and slaughter procedures

All pigs were slaughtered at the same age (26 weeks) in a commercial abattoir. Pigs from one pen were slaughtered together on the same day. The experimental design required that pigs from the two rearing systems could not be slaughtered on the same day. To avoid confounding of slaughter day and the effect of rearing system, we organised slaughter in six groups (three for conventional and three for organic pigs) with the purpose of randomizing the effect of slaughter day. Pigs were fasted for 12 hours prior to transport to the abattoir. On the day of slaughter, they were loaded between 6 and 8 a.m. and transported for 20 minutes to the local abattoir. The lairage lasted 2-3 hours. During the transport and slaughter procedures there was no mixing of the pigs. Pigs were slaughtered between 8 and 11 a.m. according to the routine abattoir procedure which consisted of CO_2 stunning (86 vol. % in air), vertical exsanguination, vapour scalding, dehairing and evisceration, followed by the veterinary inspection and SEUROP carcass classification (13).

Plasma stress markers

Stress markers were assessed in plasma taken at slaughter in order to monitor for differences in the level of stress between slaughter series. At slaughter, blood samples (approximately 4 ml) were taken into plastic tubes containing EDTA (to prevent blood

coagulation). Immediately after blood collection the tubes were placed on ice and taken to the laboratory where the blood was centrifuged at 1800 rpm for 15 minutes. The supernatants (plasma) were collected and stored at –20°C until analysis of the levels of plasma markers of stress using commercial kits. The tests were based on enzyme immunoassays for the *in vitro* diagnostic quantitative determination of cortisol, neopterin and Hsp70 according to the manufacturer's instructions (14-16). The intensity of the colour was read at 450 nm using a spectrophotometer (Varioscan Flash) and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc. Waltham, MA, USA). The concentrations of cortisol, neopterin and Hsp70 in plasma samples were expressed in µg/dL, nmol/L and ng/mL, respectively.

Carcass quality traits

At the end of the slaughter line, the pigs were classified according to SEUROP by the approved classification body (Bureau Veritas), using a method that consists of taking two measurements at the carcass split line: DM fat (minimal fat thickness over the *m. gluteus medius*) and DM muscle/width (shortest distance between the cranial end of *m. gluteus medius* and dorsal edge of the vertebral canal). The carcass lean meat percentage was calculated according to the formula (DM meat = 60.81879 – 0.72992×DM fat + 0.12157×DM muscle) approved for Slovenia (17). One day after the slaughter, additional carcass traits were measured. The hind leg (without shank) was cut off the carcass between 6^{th} and 7^{th} lumbar vertebra. It was weighed prior to and after the removal of subcutaneous fat, and the ratio between the weights was calculated. A digital image of the carcass cross section (last rib) was taken with a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). The loin eye area (*longissimus dorsi* (LD**)** area), corresponding fat area (fat over LD) and their ratio (LD meat:fat ratio) were determined from the images with LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o, Prague, Czech Republic). Belly leanness was assessed using a 1–4–7 scale (1 represents only fat, 4 half meat and half fat, and 7 only meat).

Meat quality traits

The pH was measured with a MP120 Mettler Toledo pH meter fitted with a combined glass electrode InLab427 (Mettler-Toledo, GmbH; 8603 Schwarzenbach, Switzerland) at one hour (pH_1) and 24 hours *post mortem* (pH_U). Duplicate measurements were taken in the LD muscle at the level of the last rib and in the *semimembranosus* (SM) muscle approximately 4 cm lateral to the *os pubis*. Measurements of colour CIE L* a* and b* (18) were taken one day after slaughter from a freshly cut surface of LD (at the level of the last rib) using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm diameter aperture and D_{65} illuminant, calibrated against a white tile. At the same time, the colour intensity of LD was also assessed using a 1-6 Japanese colour scale (1 and 2 represent pale, 3 and 4 normal, and 5 and 6 dark meat colour; (19)). A 2.5 cm slice of LD was taken from the loin at the level of the last rib for determination of drip loss (EZ drip loss) according to the method of Christensen (20). Drip loss was determined in duplicate, after 24 and 48 hours of storage at 4°C, and expressed as a percentage of the initial sample weight. Intramuscular fat of the LD was determined with NIR spectroscopy (NIR Systems model 6500, Silver Springs, MD, USA) according to Prevolnik *et al.* (21).

Statistical analysis

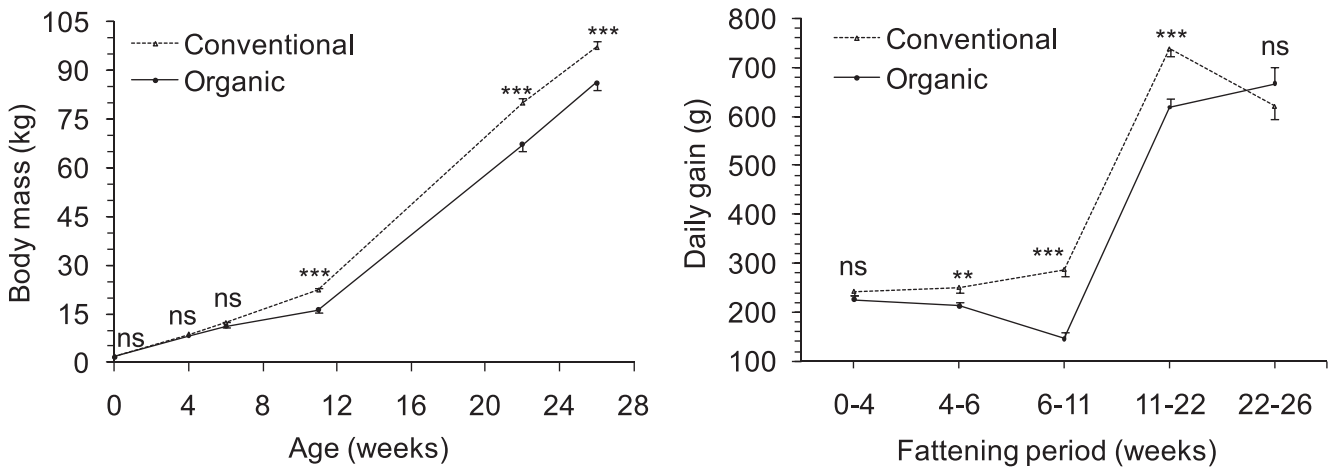
Analysis of variance (GLM procedure of SAS 9.1, SAS Inc., Cary, NC, USA) was performed in order to evaluate the effect of rearing system. For growth and carcass traits, the model comprised fixed effects of rearing system and sex, random effect of litter nested within rearing system, and interaction between rearing system and sex (which was always non-significant). For meat quality traits and plasma stress markers, an effect of slaughter day within rearing system was added to the model as a random effect. An additional analysis was performed for carcass traits, with warm carcass weight added as a covariate (in the case of meat quality traits it was non-significant). When significant effect of treatment ($P<0.05$) was detected, least squares means (*LS means*) were compared (PDIFF option, Tukey adjustment).

Results

Growth performance

Growth performance (body weight and daily gain) differed between conventional and organic pigs (Fig. 1). In Slovenia, the usual slaughter weight of pig fatteners is about 100 to 110 kg at 6 months of age and growth rate observed for pigs of conventional group

can be considered as an average result in Slovenian situation. In the first six weeks there was no difference in body weight between organic and conventional pigs. At all subsequent stages organic pigs had lower body weight than the conventional pigs. At the end of the experiment the difference in body weight between the organic and conventional pigs was 11 kg (86 vs. 97 kg, respectively), demonstrating lower growth rate of organic pigs when compared with the conventional pigs (462 vs. 523 g/day, respectively). Initially (at four weeks of age), when piglets were fed mother's milk, no difference in growth rate was observed (243 vs. 226 g/day, for conventional and organic piglets, respectively). It is only when the feed and feeding regime change that the differences are seen. Lower but non-significant growth rate of organic pigs started in weeks 4–6 when these pigs were still fed milk (the conventional pigs had been already weaned), despite being offered additional concentrate feed. Growth rate decline continued after



ns: not significant; ***P<0.001, **P<0.01.

Figure 1: Changes in body mass and daily gain (LS means ± s.e.) for pigs raised under conventional and organic rearing system

Table 3: Feed intake and feed conversion efficiency[A] of pigs raised under conventional and organic rearing system

Period	Conventional	Organic
Number of animals	32	35
4th to 6th week		
Feed intake (kg/day)	0.32	-
Feed conversion efficiency	0.781	-
6th to 11th week		
Feed intake (kg/day)	0.76	0.48
Feed conversion efficiency	0.377	0.274
Weaning to 11th week		
Feed intake (kg/day)	0.63	0.48
Feed conversion efficiency	0.439	0.274
11th to 22nd week		
Feed intake (kg/day)	1.85	1.87
Feed conversion efficiency	0.400	0.330
22nd to 26th week		
Feed intake (kg/day)	2.20	2.20
Feed conversion efficiency	0.306	0.307
Weaning to 26th week		
Feed intake (kg/day)	1.53	1.59
Feed conversion efficiency	0.383	0.336

[A]Measured per pen and calculated per pig.

the weaning (in weeks 6–11). Lower daily gain was recorded for organic pigs until week 22. In the last fattening period (weeks 22–26) organic and conventional pigs had similar daily gain (667 vs. 620 g/day, respectively). During the final period, when feed was rationed to 2.2 kg, a decrease of growth rate compared to previous phase was observed for conventional pigs, whereas this quantity was sufficient to cover the needs for increased growth rate observed in organic pigs, owing to their lower weight.

Growth performance of the pigs can be related to feed composition and feed intake (Tables 2, 3). The organic feed used in the period post-weaning had lower protein level and organic pigs exhibited lower feed intake. In other periods (weeks 11–26), feed intake and the diet were comparable between groups. Because of lower feed intake and growth rate, the organic pigs exhibited lower feed conversion efficiency.

Carcass traits

Lower growth rate of the organic pigs was reflected in most of the carcass traits (Table 4). In comparison with conventional pigs, organic pigs had lower carcass weight, lower dressing %, smaller LD muscle (DM muscle and LD area), larger fat area over LD, and lighter hams, with no significant difference in subcutaneous fat thickness (DM fat) or carcass leanness. If the influence of organic rearing is estimated for the same carcass weight, an effect is shown for fat thickness (higher than conventional), LD muscle (lower), carcass and belly leanness (lower) and LD meat:fat ratio (lower). Thus it can be speculated that organic pigs would present fatter carcasses than conventional pigs if slaughtered at the same weight.

Table 4: Carcass quality traits of pigs raised under conventional and organic rearing system[A]

	At the same age			At the same carcass weight		
	Conventional	Organic	*Sig.*	Conventional	Organic	*Sig.*
Number of animals	32	35		32	35	
Carcass weight, kg	75.8 ± 1.5	65.6 ± 2.0	***	-	-	-
Dressing, %	78.1 ± 0.5	76.2 ± 0.7	*	77.6 ± 0.5	77.2 ± 0.7	ns
DM fat, mm	14.9 ± 0.7	15.3 ± 0.9	ns	14.1 ± 0.6	16.9 ± 0.8	*
DM muscle, mm	66.7 ± 0.9	61.1 ± 1.2	***	65.5 ± 0.8	63.5 ± 1.1	ns
DM meat, %	58.1 ± 0.5	57.1 ± 0.6	ns	58.5 ± 0.4	56.2 ± 0.6	**
LD area, cm^2	44.9 ± 0.9	35.1 ± 1.2	***	43.5 ± 0.7	37.8 ± 1.0	***
Fat over LD, cm^2	13.1 ± 0.4	11.7 ± 0.5	*	12.6 ± 0.3	12.5 ± 0.4	ns
LD meat : fat ratio	3.5 ± 0.1	3.1 ± 0.1	*	3.5 ± 0.1	3.1 ± 0.1	*
Belly leanness (1-7)	5.0 ± 0.1	4.8 ± 0.2	ns	5.1 ± 0.1	4.6 ± 0.2	*
Ham, kg	9.6 ± 0.2	8.3 ± 0.3	***	9.1 ± 0.1	9.1 ± 0.1	ns
Ham in carcass, %	25.2 ± 0.2	25.4 ± 0.2	ns	25.1 ± 0.2	25.5 ± 0.2	ns
Ham meat, %	83.4 ± 0.5	82.2 ± 0.6	ns	83.7 ± 0.5	81.7 ± 0.7	*
Ham fat, %	16.6 ± 0.5	17.8 ± 0.6	ns	16.3 ± 0.5	18.3 ± 0.7	*
Ham meat : fat ratio	5.2 ± 0.2	4.7 ± 0.2	†	5.3 ± 0.2	4.5 ± 0.2	*

[A]Values in the table are LS means ± s.e.; LD - muscle *longissimus dorsi*; DM fat - minimal fat thickness over the *m. gluteus medius*; DM muscle - shortest distance between cranial end of *m. gluteus medius* and dorsal edge of vertebral canal; ns: not significant; ***P<0.001, **P<0.01, *P<0.05; †<0.10

Plasma stress markers and meat quality traits

Rearing system was not associated with any significant differences in the plasma levels of cortisol, neopterin or Hsp70 between conventional and organic pigs. However, it is worth noting that the level of Hsp70 was 2-fold higher in organic pigs.

With regard to meat quality, the rearing system had a significant effect only on pH_U and the intramuscular fat content of the LD muscle (Table 5). Although significantly higher, the difference in pH_U was small from the practical point of view and was not accompanied by any differences in colour or water holding capacity (drip loss) between organic and conventional pigs. Intramuscular fat content of the LD muscle was low in both groups of pigs, and despite lower carcass weight, the organic pigs exhibited higher intramuscular fat content than conventional pigs (1.8 vs. 1.4 %, respectively).

Table 5: Level of plasma stress markers at slaughter and meat quality traits for pigs raised under conventional and organic rearing system[A]

Item	Conventional	Organic	*Sig.*
Number of animals	32	35	
Plasma stress markers			
Cortisol, µg/dL	10.3 ± 1.1	10.7 ± 1.3	ns
Neopterin, nmol/L	3.0 ± 0.5	2.8 ± 0.6	ns
Hsp70, ng/mL	3.6 ± 1.4	7.4 ± 1.6	†
Meat quality traits			
SM pH_1	6.30 ± 0.06	6.44 ± 0.10	ns
SM pH_U	5.64 ± 0.03	5.66 ± 0.04	ns
LD pH_1	6.03 ± 0.08	6.17 ± 0.12	ns
LD pH_U	5.52 ± 0.01	5.58 ± 0.02	**
LD colour (1-6)	3.6 ± 0.1	3.6 ± 0.1	ns
Minolta L*	54.1 ± 0.5	54.4 ± 0.7	ns
Minolta a*	6.1 ± 0.2	6.2 ± 0.3	ns
Minolta b*	2.7 ± 0.1	2.8 ± 0.2	ns
Drip loss 24h , %	4.3 ± 0.5	4.9 ± 0.7	ns
Drip loss 48h, %	6.4 ± 0.5	7.2 ± 0.7	ns
Intramuscular fat, %	1.40 ± 0.10	1.77 ± 0.13	*

[A]Values in the table are LS means ± s.e.; SM - muscle *semimembranosus*; LD - muscle *longissimus dorsi*; pH_1 - pH measured one hour after slaughter; pH_U - pH measured 24 hours after slaughter; ns - not significant; **P<0.01, *P<0.05; † P<0.10; Frequency of PSE meat (fast pH fall shortly *post-mortem*) was 18% in conventional and 20% in organic pigs

Discussion

Growth performance

The lower growth rate of the organic pigs started during the last stage of lactation and persisted until 22 weeks of age. To explain growth retardation several factors should be considered, which may have interacted, the prolonged lactation, the lower post-weaning feed intake (due to less palatable organic feed or its lower protein level) and larger space provided to organic pigs, which will be discussed below.

The observed growth retardation in weeks 4-6 can be related to the prolonged lactation of organic pigs, which results from the diminished milk yield of the sows at the end of lactation, although all the pigs were supplied with additional concentrate. The most noticeable decrease of growth rate in organic pigs was observed post-weaning and it coincides with lower feed intake of organic pigs. The importance of feed intake for growth traits has previously been demonstrated (22). A possible reason for the lower feed intake, given that we observed no particular problem with diarrhoea in the organic pigs, could be the lower palatability of the organic feed mixture (1). It is also possible that the animals felt satiated earlier on the organic diet. The regulation of the appetite is a complex mechanism which acts via ghrelin, a 28-amino acid peptide. Ghrelin is involved in tryptophan-mediated appetite stimulation in swine (23) and has been shown to be stimulated by protein ingestion in men (24,25). Deficiency of tryptophan has been shown to reduce appetite and feed intake in pigs (26,27).

Moreover, the organic feed concentrate used for organic pigs in the growing phase had lower concentrations of protein and lysine than the conventional diet (Table 2), which is critical in the first stage of fattening. The availability of essential amino acids adequate for growth is known to be the main difficulty in organic feeding of pigs (28). Organic standards, and especially the ban on synthetic amino acids, make it difficult to meet the amino acid requirements of young pigs. Organic sources rich in amino acids and in lysine could be produced, but are expensive and therefore rarely produced and used for economical reasons. In the present study, commercially available diet was used containing 8.0 g/kg of lysine (Table 2), whereas according to Sundrum (29) requirements for suckling piglets and weaners can be covered with diets containing at least 12 g lysine/kg.

Underfeeding or nutrient deprivation during the post-weaning period has a major impact on subse-

quent growth performance (30). Accelerated growth following a period of slower growth, as a result of restricted feeding or nutrient deprivation, is defined as compensatory growth. The use of feeding strategies for obtaining compensatory growth in organic pigs has been of interest (11,31), especially because of the problems with protein supply in organic pig production.

In the finishing period (weeks 22–26) the growth rate of the organic and conventional pigs was comparable. Requirements for amino acids, especially lysine, decrease as animals get older and heavier (32), and the lysine content of both diets in the later phases of this experiment was sufficient (29). In the finishing period the organic pigs received the same quantity of feed, and of similar composition, as the conventional pigs, which was sufficient to cover the needs for increased growth rate of organic pigs due to lower body weight.

Due to lower feed intake, the organic pigs had lower feed conversion efficiency. However, we cannot exclude the possibility that feed efficiency might have been affected by other parameters such as feed composition, pen space, and outdoor access. The greater physical activity of pigs, which were allocated a larger space and access to an outdoor area, may have resulted in higher energy expenditure.

Comparison of the results of the present study with similar literature reports (studies using feeding of organic concentrates) shows inconsistency. In agreement with our results, Enfält *et al.* (6) observed a delay in reaching slaughter weight for pigs grown under organic conditions with outdoor access, as compared to conventional pigs, although in their study pigs were fed *ad libitum* throughout the experiment and had more outdoor space as in the present study. On the contrary, Millet *et al.* (8) observed a better growth rate for pigs in organic housing that were fed organic feed concentrate *ad libitum,* as compared to conventional pigs. Several other studies have also reported a higher feed intake and growth rate for organic pigs, as compared to conventional pigs (1,2,10). Strudsholm and Hermansen (11) reported that when pigs had been fed *ad libitum* with concentrates, indoor compared with outdoor rearing did not affect growth rate, only feed consumption (which increased). The value of the present study is a demonstration of possible problems with commercially available organic feed mixtures for piglets in early life, which was reflected in growth performance.

Carcass quality

Due to slower growth of organic as compared to conventional pigs, carcass weight and consequently other carcass traits were affected. Differences between the organic and conventional pigs were observed mainly for the traits of muscularity, for which the most influential explanatory factors seem reduced growth rate due to lower feed intake and limited supply of proteins in organic feed concentrate. The comparison between organic and conventional pigs simulated for the same carcass weight indicated that organic pigs would be fatter if slaughtered at the same weight as conventional pigs. This result can be related to the compensatory growth of organic pigs affecting the deposition of adipose rather than muscle tissue.

Published studies dealing with pig carcass traits under organic production regimes (1,2,6,8,10,11) show significant effects of housing and feeding, but the results are difficult to compare and inconsistent since they reflect the variability in the feeding and housing systems applied in different studies. One study (2) however can be highlighted which showed no difference in carcass quality when organic and conventional pigs were reared *ad libitum*, while 70% restriction of concentrates given to organic pigs reduced their growth rate and increased the lean meat percentage.

Plasma stress markers and meat quality

In the present experiment the main purpose of assessing plasma stress markers at slaughter was to monitor for the effect of slaughter day (and pre-slaughter susceptibility to stress), owing to its importance for meat quality. The biochemical markers used (cortisol, neopterin and Hsp70) reflect different aspects of stress. Cortisol is the main hormone of hypothalamic–pituitary–adrenocortical axis and is released by the adrenal cortex in response to stress. It influences feeding behaviour, pancreatic hormone secretion, energy expenditure, and protein/lipid balance (33). In the present study, plasma cortisol levels at slaughter were similar in pigs from both rearing systems. The comparable level of cortisol indicates a similar stress response to the pre-slaughter procedures for both rearing systems. Studies that deal with the cortisol level in different rearing systems are rare. In accordance with our results, Barton-Gade (7) and Lebret *et al.* (28) reported no effect of rearing system on the plasma

cortisol level prior to slaughter. A comparable level of neopterin in organic and conventional pigs is an indicator of similar health status, because studies suggest it is a good marker of cellular immune activation, with increased concentrations detected in infections, autoimmune diseases and animals with tumours (34). Hsp70 is a stress-limiting factor that is involved in the response to stress at a cellular level (35). The results of the present study suggest that rearing system was not associated with plasma Hsp70 level. Despite this, it is interesting to observe that the level of Hsp70 was 2-fold higher in organic pigs. A possible explanation for the lack of significance was the high variation within groups. Nevertheless, we can consider this result indicative of a stronger response of the organic pigs to stress in terms of cell protein protection.

Regarding meat quality an effect of rearing system was observed only for pH_U and LD intramuscular fat content, both being higher in organic pigs. Differences in pH_U were not big enough to be reflected in LD colour or drip loss, which are properties important for the consumer, and highly correlated to pH_U (36). In contrast to our results, higher pH_U values in conventional as compared to organic pigs (6,8), or no differences (2,9), have been reported. Studies show, that intramuscular fat is higher in older and heavier pigs and follows general body adiposity (37,38). Despite a considerably lower carcass weight, higher intramuscular fat was observed in organic pigs in the present study, which can be related to feeding. A strategy of restriction and re-alimentation has been suggested to increase intramuscular fat content (38). Contrary to our result, organic feeding has often been shown to reduce intramuscular fat (1,6,10), or no effect has been observed (2). On the whole, the results of the present study corroborate previous reports which show limited effect of organic production on meat quality.

Conclusions

In comparison to conventional rearing, in the present study, organic pigs exhibited post-weaning growth retardation and as a consequence lower carcass quality. The increased growth rate of organic pigs in the last fattening phase, following the initial growth retardation, explains lower carcass quality and higher intramuscular fat content, the latest being of interest in terms of improved meat quality. The results of the present study indicate possible problems associated with the use of commercially available organic diets for piglets.

Acknowledgements

The authors acknowledge the financial support by Slovenian Research Agency (project J4-9532, project V4-0338, program P4-0072) and partly by the Ministry of Agriculture Forestry and Food.

References

1. Millet S, Hesta M, Seynaeve M, et al. Performance, meat and carcass traits of fattening pigs with organic versus conventional housing and nutrition. Livest Prod Sci 2004; 87: 109–19.

2. Hansen L, Claudi-Magnussen C, Jensen S, Andersen H. Effect of organic pig production systems on performance and meat quality. Meat Sci 2006; 74: 605–15.

3. Chang J, Lusk J. Fairness and food choice. Food Policy 2009; 34: 483–91.

4. Bornett H, Guy J, Cain P. Impact of animal welfare on costs and viability of pig production in the UK. J Agric Environ Ethics 2003; 16: 163–86.

5. Council Regulation (EC) No 889/2008 of 5 Septembre laying down detailed rules for the implementation of Council Regulation (EC) no 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Off J Eur Union L 2008; 51(250): 1-84.

6. Enfält A, Lundström K, Hansson I, Lundeheim N, Nyström P. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. Meat Sci 1997; 45: 1–15.

7. Barton–Gade P. Effect of rearing system and mixing at loading on transport and lairage behaviour and meat quality: comparison of outdoor and conventionally raised pigs. Animal 2008; 2: 902–11.

8. Millet S, Raes K, Van den Broeck W, De Smet S, Janssens G. Performance and meat quality of organically versus conventionally fed and housed pigs from weaning till slaughtering. Meat Sci 2005; 69: 335–41.

9. Oksbjerg N, Strudsholm K, Lindahl G, Hermansen JE. Meat quality of fully or partly outdoor reared pigs in organic production. Acta Agric Scand 2005; 55: 106–12.

10. Olsson V, Andersson K, Hansson I, Lundström K. Differences in meat quality between organically and conventionally produced pigs. Meat Sci 2003; 64: 287–97.

11. Strudsholm K, Hermansen J. Performance and carcass quality of fully or partly outdoor reared

pigs in organic production. Livest Prod Sci 2005; 96: 261–8.

12. Zakon o zaščiti živali = Law on Animal Protection. Uradni List RS 2007; 17(43): 5943

13. Commission Regulation (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcasses and the reporting of prices thereof. Off J Eur Union L 2008; 51(337): 3–30.

14. IBL International. Cortisol ELISA: enzyme immunoassay for the in-vitro-diagnostic quantitative determination of free cortisol in human saliva and total cortisol in dilutet serum. RE52611: instruction for use 2008. Hamburg: IBL International GMBH, 7 str.

15. Assay designs. Hsp70 higs sensitivity EIA kit: enzyme immunoassay kit for quantitative determination of Hsp 70 in human serum and plasma. EKS-715: instruction for use 2008. Hamburg: IBL International GMBH, 16 str.

16. IBL International. Neopterin ELISA: enzyme immunoassay for the in-vitro-diagnostic quantitative determination of neoptherin in human serum, plasma and urine. RE59321: instruction for use 2008. Hamburg: IBL International GMBH, 6 str.

17. Commission decision of 18 February 2008 amending Decision 2005/879/EC authorising methods for pigs carcases in Slovenia. Off J Eur Union L 2008; 51(56): 28–30.

18. CIE. Recommendations on uniform color spaces-color difference equations, psychometric color terms. International Commission on Illumination, CIE Publication No.15, Supplement 2, 1978.

19. Nakai H, Saito F, Ikeda T, Ando S, Komatsu A. Standard models of pork colour. Bull Nat Inst Anim Ind 1975; 29: 69–74.

20. Christensen L. Drip loss sampling in porcine m. longissimus dorsi. Meat Sci 2003; 63: 469–77.

21. Prevolnik M, Čandek-Potokar M, Škorjanc D, et al. Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. JNIRS 2005; 13: 77–85.

22. Arthur P, Giles L, Eamens G, Barchia I, James K. Measures of growth and feed efficiency and their relationships with body composition and carcass traits of growing pigs. Anim Prod Sci 2009; 49: 1105–12.

23. Zhang H, Yin J, Li D, Zhou X, Li X. Tryptophan enhances ghrelin expression and secretion associated with increased food intake and weight gain in weanling pigs. Domest Anim Endocrinol 2007; 33: 47–61.

24. Erdmann J, Lippl F, Schusdziarra V. Differential effect of protein and fat on plasma ghrelin levels in man. Regul Pept 2003; 116: 101-7.

25. Erdmann J, Topsch R, Lippl F, Gussmann P, Schusdziarra V. Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. J Clin Endocrinol Metab 2004; 89: 3048-54.

26. Eder K, Peganova S, Kluge H. Studies on the tryptophan requirement of piglets. Arch Anim Nutr 2001; 55: 281–97.

27. Seve B, Le Floc'h N. Mutual valorisation of crystalline L-tryptophan and L-threonine in starter feed for piglets. Role of the liver threonine dehydrogenase. J Rech Porcine Fr 1998; 30: 209–16.

28. Lebret B, Meunier-Salaun MC, Foury A, Mormede P, Dransfield E, Dourmad JY. Influence of rearing conditions on performance, behavioral, and physiological responses of pigs to preslaughter handling, carcass traits, and meat quality. J Anim Sci 2006; 84: 2436–47.

29. Sundrum A. Managing amino acids in organic pig diets. In: Hovi M, Baars T, eds. Proceedings of 4th NAHWOA Workshop. Wageningen, 2001: 181–91.

30. Le Dividich J, Sève B. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domest Anim Endocrinol 2000; 19: 63–74.

31. Fernández J, Nørgaard J. Compensatory growth in slaughter pigs reared under organic conditions. J Sci Food Agric 2009; 89: 1376–81.

32. van Milgen J, Valancogne A, Dubois S, Dourmad J, Sève B, Noblet J. InraPorc: a model and decision support tool for the nutrition of growing pigs. Anim Feed Sci Technol 2008; 143: 387–405.

33. Dallman MF, Strack AM, Akana SF, et al. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 1993; 14: 303–47.

34. Breineková K, Svoboda M, Smutná M, Vorlová L. Markers of acute stress in pigs. Physiol Res 2007; 56: 323–29.

35. Liu Y, Steinacker JM. Changes in skeletal muscle heat shock proteins: pathological significance. Front Biosci 2001; 6: 12–25.

36. Prevolnik M, Čandek-Potokar M, Novič M, Škorjanc D. An attempt to predict pork drip loss from pH and colour measurements or near infrared spectra using artificial neural networks. Meat Sci 2009; 83: 405–11.

37. Čandek-Potokar M, Žlender B, Lefaucheur L, Bonneau M. Effects of age and/or weight at slaughter on longissimus dorsi muscle: biochemical traits and sensory quality in pigs. Meat Sci 1998; 48: 287–300.

38. Lebret B. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. Animal 2008; 2: 1548–58.

RASTNOST, KLAVNE LASTNOSTI IN KAKOVOST MESA PRAŠIČEV, VZREJENIH V EKOLOŠKEM ALI KONVENCIONALNEM NAČINU REJE Z UPORABO KOMERCIALNO DOSTOPNIH KRMNIH MEŠANIC

M. Prevolnik, M. Ocepek, M. Čandek-Potokar, M. Bavec, D. Škorjanc

Povzetek: Cilj raziskave je bil oceniti rastnost pujskov od rojstva do zakola, klavne lastnosti in kakovost mesa prašičev, vzrejenih bodisi konvencionalno (n = 32) ali upoštevajoč standarde ekološke reje (n = 35) ob uporabi ekoloških krmnih mešanic, ki so na voljo na trgu. V tej raziskavi je bila poraba krme od odstavitve do 11. tedna za 22 % nižja pri prašičih iz ekološke reje. Posledično so ekološko vzrejeni prašiči imeli nižje priraste vse do 22. tedna starosti. V zadnji fazi pitanja (od 22. do 26. tedna), ko je bila razpoložljiva krma omejena, se je prirast pri konvencionalnih pitancih zmanjšal, pri ekoloških pa povečal. Vsi prašiči so šli v zakol pri običajni komercialni starosti (26 tednov). Zaradi počasnejše rasti so imeli ekološko vzrejeni prašiči manjšo maso trupa, manjši klavni izplen, manjšo mišico *longissimus dorsi* (LD) in lažja stegna, medtem, ko pri debelini podkožne maščobe in mesnatosti trupov (z izjemo površine podkožne maščobe nad LD) ni bilo razlik. Ob korekciji na maso trupov je analiza pokazala, da bi ekološki prašiči imeli bolj zamaščene trupe kot konvencionalni, če bi bili zaklani pri enaki masi. V zvezi s kakovostjo mesa je bila pri ekološko vzrejenih prašičih ugotovljena višja vrednost pH po 24 urah in višja vsebnost mišične maščobe. Rezultati kažejo, da pitanje prašičev z uporabo komercialno dostopnih ekoloških krmnih mešanic za pujske lahko vodi v slabše proizvodne rezultate (manjše zaužitje krme, nižji prirasti), po drugi strani pa lahko takšna dieta poveča vsebnost mišične maščobe, ki je zanimiva zaradi izboljšanja kakovosti mesa.

Ključne besede: ekološka reja; rast; klavna kakovost; kakovost mesa; stres; prašič

Slov Vet Res 2011; 48 (1): 27-34
UDC 636.5.083:636.087.7:637.51

Original Scientific Article

EFFECT OF SUPPLEMENTATION OF PHYTOGENIC FEED ADDITIVES ON PERFORMANCE PARAMETERS AND MEAT QUALITY OF BROILER CHICKENS

Slavomír Marcinčák[1]*, Peter Popelka[1], Nevijo Zdolec[2], Mária Mártonová[1], Jana Šimková[1], Dana Marcinčáková[1]

[1]University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81, Košice, Slovak Republic; [2]Department of Hygiene and Technology of Foodstuffs of Animal Origin, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

*Corresponding author, E-mail: marcincak@uvm.sk

Summary: The aim of the experiment was to study the effects of clove powder *(Syzygium aromaticum* L*)*, extracts of agrimony (*Agrimonia eupatoria* L.) and lemon balm (*Melissa officinalis* L.) on broiler chicken performance, carcass and meat quality. Three treatment combinations were prepared: C – control group without any feed or water additive; AC – feed supplemented with clove powder and drinking water supplemented with agrimony extract; LC – feed supplemented with clove and drinking water supplemented with lemon balm extract. AC group chickens had significantly higher body weight ($P < 0.05$) compared to the control group within 14 days of fattening. On the 42nd day higher body weight, better feed conversion, higher carcass yield and higher breast and thigh weight were determined in broiler chickens from groups AC and LC. However, significant differences ($P > 0.05$) in selected parameters were not recorded in experimental groups AC and LC compared to control. Dry matter of thigh muscles derived from AC and LC broiler chickens were significantly higher ($P < 0.05$) compared to controls. In groups AC and LC lower total fat content was recorded in thigh muscles ($P > 0.05$) when compared with control and significantly higher ($P < 0.05$) proportion of total protein was found in AC group. In the sensory evaluation of meat experimental groups AC and LC were rated better, despite no significant differences ($P > 0.05$) in comparison with control group.

Keywords: agrimony; clove; growth of broilers; lemon balm; meat quality

Introduction

A ban on the use of antibiotics as growth promoters in the European Union in 2006 due to increase bacterial resistance in human population encouraged the search for replacement alternatives, which would be useful to improve the production parameters of fattening pigs and poultry (1). A new group of potential feed additives are herbs, their extracts or essential oils (2).

Phytogenic feed additives are usually defined as products derived from plants and added to animal feed for fattening to increase productivity, improving the quality of feed and animal hygiene conditions and not least to improve the quality of produced food. Feed additives produced from plants have often a significant antibacterial effect, thereby suppressing pathogenic microflora in the gastrointestinal tract of animals and thus reducing mortality during the fattening period, especially in stress period (3). Plant additives are often added into feedstuff as they improve the taste and smell of feed and thus improve intake and growth of animals (1). Several herbal additives contain substances which increase the production of digestive juices (saliva, gastric juices, pancreatic and intestinal secretion) and thereby enhance appetite and digestion (4).

Several plants or their essential oils, especially those with aromatic character such as cloves, rosemary, cinnamon (1), anise (5), but also oregano (6) and sage (7) have been used in fattening. Plant ar-

Received: 17 January 2011
Accepted for publication: 24 February 2011

omatic oils as well as probiotics and organic acids can be used to support growth in order to achieve improvements in breeding broilers in organic farming system (8). Supplementation of feed or drinking water with plant aromatic oils increases body weight of chickens and favourably has an effect on feed conversion (9).

For a wider range of action the most suitable combination of extracts is researched. The role of research is to find the most effective concentrations and combinations of plants and essential oils. According to Ertas et al. (10), the combination of some plants extracts has a better effect on the growth performance in poultry than their individual supplementation. Synergism between some herbal constituents was highlighted in the studies of Hernandez et al. (7) and Burt (11).

Clove (*Syzygium aromaticum* L.) is considered as one of the most versatile herbs due to its analgesic and anti-inflammatory, anaesthetic, antimicrobial and antifungal effect (12), antiseptic, appetite and digestion stimulation (13), anti-inflammatory, anticarcinogenic, antiparasitic as well as antioxidant properties (6, 14, 15). The major constituent of clove is an essential oil (up to 20 %), which is characterized by presence of up to 85.5 % of eugenol (16). Lemon balm (*Mellisa officinalis* L.) and agrimony (*Agrimonia eupatoria* L.) are the most common herbs used in our traditional folk herbal medicine (17). However, little is known about the antioxidant properties of their extracts and essential oils in poultry. The alcohol extracts of both herbs are a potent source of polyphenols (6, 17, 18). In *in vitro* experiments, it was found that active substances extracted from agrimony possess a significant radical scavenging activity as well as potential antioxidant capacity (18, 19).

The aim of our work was to study the effect of combination of cloves added to the feed and agrimony or lemon balm extracts administered into water on growth of broiler chickens and quality parameters of produced meat.

Materials and methods

Plant material

Pulverized extracts of agrimony (*Agrimonia eupatoria* L.) and lemon balm (*Mellisa officinalis* L.) were prepared by Calendula (Nova Bana, Slovak Republic) and both herbs were collected in East Slovakia. The plant material consisted of leaves, flowered tops and stalks that was dried at 30 - 35 °C, ground, extracted with 50 % ethanol and evaporated to obtain a powder (prescription and protocol of Calendula, Slovak Republic). Clove (*Syzygium aromaticum* L.) powder was purchased from Mäspoma (Zvolen, Slovak Republic).

Experimental animals, diets and treatments

The experiment was carried out on 120 one-day-old unsexed hybrid broiler chickens Ross 308 which were randomly allotted to 3 groups (40 birds per group). Broilers were kept in large pens with wood shavings. On the day of hatching, the initial room temperature was 32 °C. It was gradually decreased by 3 °C weekly to a final temperature of 23 °C on the 21st day and then kept constant. During the entire fattening period, the lighting regimen was 24 h of continuous light per day. Broilers were reared in the air environment with 70 % humidity. The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic.

All birds were fed with commercial basal diets (BD) for broilers “Starter” for days 1 to 14, “Grower” for days 15 to 29 and “Finisher” for days 30 to 42. The composition of all BDs is presented in Table 1. The control group of chickens received the basal diet (BD) only. The second (LC) group was fed with the same BD enriched with 1 % clove buds powder combined with 0.2 % lemon balm extract diluted in drinking water for 42 days. Third group (AC) was also fed with BD enriched with 1 % clove buds powder combined with 0.2 % agrimony extract diluted in drinking water. The broilers had a free access to the feed and water.

Data collection and analysis

Body weights were recorded weekly. Feed intake per group was measured throughout the experiment and the feed conversion ratio was calculated at the end of experiment. Broiler chickens were slaughtered on the 42nd day of fattening. After the carcass processing, chickens in each group were weighed, boned and chilled at 4 °C. The yield of carcasses was calculated by dividing the body weight of the animal before slaughter and carcass weight after evisceration. The proportion of breast and thigh muscle was calculated from a weight of individual parts and body weight after evisceration.

Chemical composition of meat samples

Determination of water content, dry matter content and fat content in % was performed according to Veterinary laboratory methods (20).

Sensory evaluation

For sensory analysis of meat samples thigh and breast muscle were packed into containers and evaluated 24 hours after slaughtering. Professional evaluation committee was represented by a panel of 7 assessors who worked according to Methods intended for meat sensory evaluation (21). The samples were boiled and 5-point scheme was used while the maximum number of evaluation points was 20 (22).

Table 1: Composition of basal diets given to the broilers during the entire experiment

Ingredients (%)	Starter (1 to 14 day)	Grower (15 to 29 day)	Finisher (30 to 42 day)
Maize	51.3	49.0	52.8
Wheat	8.0	10.0	20.0
Wheat meal	7.0	4.00	-
Soybean meal (46.5 % CP, 1.5 % fat)	29.9	31.6	23.5
Wheat bran	-	2.25	-
Limestone	1.90	1.25	1.75
Monocalcium phosphate	0.89	1.00	0.90
Vitamin-mineral premix [a]	0.30	0.3	0.30
NaCl	0.36	0.30	0.35
L-lysine	0.25	0.15	0.15
DL-methionine	0.10	0.15	0.25
Nutrient level (%)			
Linoleic acid	1.0	1.0	1.0
Metabolizable energy (MJ/kg)	11.5	12.0	12.0
Crude protein	17.5	19.0	17.0
Crude fibre	5.0	4.0	4.0
Ash	80.0	70.0	70.0
L-lysine	0.80	0.95	0.95
DL-methionine	0.35	0.40	0.40
Methionine + cysteine	0.70	0.75	0.70
Calcium	0.80	0.70	0.70
Phosphorus	0.50	0.50	0.50

[a] supplied per kg of basal diet: vitamin A 8,000,000 IU; vitamin D_3 1,200,000 IU; vitamin E 15,000 mg; vitamin K_3 3,000 mg; vitamin B_1 1,500 mg; vitamin B_6 8,000 mg; niacin 15,000 mg; choline chloride 50,000 mg; pantothenic acid 50 mg; pyridoxine 5 mg; folic acid 2 mg; cyanocobalamine 30 μg; biotin 0.2 mg; I 2 mg; Co 1 mg, K 8.6 g; Cl^- 2 g; Cu 6.0 mg; Fe 60 mg; Zn 50 mg; Mn 50 mg

Statistical analysis

Statistical treatment of results was performed using statistical program GraphPad Prism, version 4.00 (23). Results are expressed as arithmetic mean (x) and standard deviation (sd). Results in each group were compared with each other by one-way ANOVA test. To compare the statistical differences between values Tukey's comparison test was used and $P < 0.05$ was regarded as statistically significant.

Results

Average weights of chickens during the whole fattening period are shown in Table 2. The addition of clove (1%) in the feed and agrimony extract (0.2%) into water had the greatest impact on weight of broiler chickens at the beginning of fattening period. At the age of 14 days, the weight of chickens was significantly higher compared with control ($P < 0.05$). On the 28th and 42nd day, the weights of both

experimental groups were higher but no significant differences were recorded when compared with control (P > 0.05). However, chickens in experimental groups were balanced and were seen less fluctuation in weight than control. The total feed consumption in all three groups was approximately the same (Table 2, P > 0.05). Feed conversion, i.e. conversion of feed to gain 1 g, was lower in both experimental groups in comparison with control. The best values were seen in group AC (1.83).

Carcass weight in all groups was correlated with weight of living animals before slaughter (Table 3). The average weights of carcasses from experimental groups were higher compared to controls (P > 0.05). The highest average weight of carcasses and also carcass yield percentage was recorded in experimental group AC supplemented with 1% clove in feed and 0.2% extract of agrimony in water. Highest average weights of thigh and breast muscles were recorded in experimental group AC (P > 0.05) again.

Table 2: Effect of supplementation of combination of clove (*Syzygium aromaticum* L) and agrimony (*Agrimonia eupatoria* L) or lemon balm (*Melissa officinalis* L) on broiler performance

Parameters	Treatments		
	C	LC	AC
LBW 0, g	41 ± 4	42 ± 3	41± 4
LBW14, g	253 ± 13a	260 ± 16ab	270 ± 18b
LBW 28, g	1025 ± 133	1046 ± 104	1118 ± 129
LBW 42, g	2112 ± 262	2156 ± 222	2232 ± 172
ADWG, g	49.30	50.33	52.16
FC 1 – 42, g (g)	4108	4137	4086
FCR (0 – 42)	1.94	1.91	1.83

C – control, LC – Clove (1 %) + Lemon balm (0.2 %), AC – Clove (1 %) + Agrimony (0.2%), LBW– live body weight, ADWG – average daily weight gain, FC – feed consumption, FCR – Feed conversion ratio.
[a,b] – values with different labelling in row are statistically different.

Table 3: Effect of suplementation of combination of clove (*Syzygium aromaticum* L) and agrimony (*Agrimonia eupatoria* L) or lemon balm (*Melissa officinalis* L) on slaughter characteristics and meat cat-ups

	C	LC	AC
Final body weight (g)	2112 ± 262	2156 ± 222	2232 ± 172
Carcass yield (g)	1449 ± 231	1473 ± 109	1558 ± 149
Carcass yield (%)	68.60	68.32	69.80
Breast (g)	294 ± 52	303 ± 31	342 ± 33
Breast yield (%)	20.29	20.57	21.95
Thighs (g)	364 ± 44	370 ± 35	410 ± 27
Thighs (%)	25.55	25.52	26.31

C – control, LC – Clove (1 %) + Lemon balm (0.2%), AC – Clove (1 %) + Agrimony (0.2%)

Results of chemical composition and sensory analysis of breast and thigh muscles are shown in Table 4. Added extracts had no effect on the chemical composition of breast muscle and experimental groups were comparable with control. Only a smaller proportion of crude protein (P > 0.05) was recorded in the breast muscles of experimental groups. Dry matter content of thigh muscle was significantly lower in the experimental groups (AC, LC) compared with control (P < 0.05). In the experimental group AC a significantly higher proportion of crude protein was recorded compared to other groups (P < 0.05). Lower proportion of fat in thigh muscles was analyzed in experimental groups (AC, LC) compared with control (P > 0.05). Adding plant additives to feed had also a positive impact on the sensory evaluation of breast and thigh muscle (Table 4). Breast and thigh muscles of experimental groups were scored better in comparison with control (P > 0.05). Breast muscle samples were evaluated as the best after feeding cloves and lemon balm extract (LC). As for thigh muscle the best evaluation of a sample was seen after feeding the cloves and agrimony extract (AC).

Table 4: Chemical composition and sensory evaluation of meat

Treatments		Dry matter	Crude fat	Crude protein	Sensory evaluation
C	Breast	25.76 ± 054	1.60 ± 0.45	23.11 ± 0.14	15.40 ± 1.63
	Thigh	28.37 ±0.27[a]	9.34 ± 0.20	17.59 ± 0.02[a]	16.80 ± 1.90
LC	Breast	25.65 ± 0.28	1.75 ± 0.31	22.91 ± 0.14	16.60 ± 2.01
	Thigh	27.51 ± 0.11[b]	8.80 ± 0.13	17.70 ± 0.07[a]	16.82 ± 1.70
AC	Breast	25.89 ± 0.23	1.65 ± 0.35	22.88 ± 0.17	15.87 ± 1.68
	Thigh	27.12 ± 0.25[b]	8.75 ±0.70	17.90 ± 0.09[b]	17.60 ± 1.63

C – control, LC – Clove (1 %) + Lemon balm (0.2%), AC – Clove (1 %) + Agrimony (0.2%)
[a,b] – values with different labelling in column are statistically different.

Discussion

In recent years, an interest in plant feed additives as alternative growth a promoter has increased because of the prohibition of the use of antibiotic feed additives. Plant (phytogenic) growth promoters act primarily as regulators of intestinal flora suppressing the growth of potential pathogens in the intestinal tract, especially in critical period of stress (1, 2). In poultry critical period is mainly at the beginning of feeding, which can result in reduced growth and mortality of animals (24). Even in our experiment, the most significant increase in weight was achieved for 14 days of fattening. On the 28th and the 42nd day, the weight of both experimental groups was higher but no significant differences were observed when compared with control ($P > 0.05$). The effect of plants and their essential oils on the final weight of chickens has been described in several works (1, 2, 4, 5, 7, 10). However, their effect on increasing total weight of chickens is inconsistent (1). The resulting effect depends on plant extracts used in feeding and on their proper concentrations. Adding only one plant extract to the feed or water does not favourably influence the growth parameters of poultry (4). This problem could be overcome by a combined supplementation of different herb constituents with the synergistic effect (2, 8, 25). Hernandez et al. (7) indicate higher weight gains of chickens after feeding a combination of sage, thyme and rosemary extracts in a dose of 5 g per kilogram of feeding mixture. After feeding a combination of these extracts feed conversion was also decreased by 4%.

Equal or lower consumption of feed during fattening after addition of plants essential oils is mentioned by several authors (2, 26). Although even in these indicators are not uniform scientific results, generally there is a perception that plant additives have neutral or positive impact on consumption and feed conversion (1, 2, 24).

Higher carcass yield was also recorded after the addition of extracts of coneflower, thyme (27) in feed, and in a combination of *Nigella sativa* extract in feed and coneflower extract administered in water (28). Even in our experiment, higher carcass weight and higher weight of breast and thigh muscles were recorded in experimental groups (AC, LC). Although differences in weight are not statistically significantly higher when compared with control, weight of carcass, breast and thigh muscles of poultry fed with cloves in feedstuff and agrimony extract in water may be interesting for poultry producers.

Plumbless importance of plant feeding additives is also in terms of quality of produced meat. Most of the plants and their extracts have strong antioxidant properties, significantly resulting in lower fat oxidation during storage of meat (29). Added plant additives often have a positive effect on the sensory evaluation of produced meat (2). Our results showed that feeding a combination of cloves in the feed and agrimony or lemon balm extracts in water to broiler chickens had a slightly positive effect on the sensory evaluation of produced meat. Several authors mention better sensory characteristics of poultry meat after adding plants with antioxidant activity (25, 30). Positively improved taste and smell were evaluated in meat from chickens supplemented with rosemary powder (31).

Acknowledgements

The work was carried out with the financial support of grant project VEGA 1/0648/11.

References

1. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 2008; 86: 140-8.

2. Brenes A, Roura E. Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol 2010; 158: 1-14.

3. Schone, F. Vetter A, Hartung H, et al. Effects of essential oils from fennel (*Foeniculi aetheroleum*) and caraway (*Carvi aetheroleum*) in pigs. J Physiol Anim Nutr 2006; 90: 500-10.

4. Barreto MSR, Menten JFM, Racanici AMC, Pereira PWZ, Rizzo PV. Plant extracts used as growth promoters in broilers. Braz J Poultry Sci 2008; 10: 109-15.

5. Al-Kassie GAM. The effect of anise and rosemary on broiler performance. Int J Poultry Sci 2008; 7: 243-5.

6. Marcincak S, Popelka P, Šoltysová L. Polyphenols and antioxidative activity of extracts from selected slovakian plants. Acta Sci Pol, Med Vet 2008; 7: 9-14.

7. Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD. Influence of two plant extracts on broiler performance, digestibility and digestive organ size. Poultry Sci 2004; 83: 169-74.

8. Alcicek A, Bozkurt M, Cabuk M. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South Afr J Anim Sci 2004; 34: 217-22.

9. Basset R. Oregano´s positive impact on poultry production. World Poultry 2000; 16: 31-4.

10. Ertas ON, Guler T, Ciftci M, Dalkilic B, Simsek UG. The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Int J Poultry Sci 2005; 4: 879-84.

11. Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. Int J Food Microbiol 2004; 94: 223-53.

12. Dalkilic B, Guler T. The effects of clove extract supplementation on performance and digestibility of nutrients in boilers. F U Sag Bil Vet Derg 2009; 23: 161-8.

13. Kamel KC. Tracing modes of action and the roles of plant extracts in non-ruminants. In: Garnsworthy PC, Wiseman J, eds. Recent advances in animal nutrition. Nottingham: University Press, 2001: 135-50.

14. Dorman HJD, Surai P, Deans SG. *In vitro* antioxidant activity of a number of plant essential oils and phytoconstituents. J Essent Oil Res 2000; 12: 241-8.

15. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr 2003; 133: 1286-90.

16. Viuda-Martos M, Navajas YR, Zapata ES, Fernández-López J, Pérez-Álvarez JA. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Fragrance J 2010; 25: 13-9.

17. Heilerová Ľ, Bučková M, Tarapčík P, Šilhár S, Labuda J. Comparison of antioxidative activity data for aqueous extracts of lemon balm, oregano, thyme and agrimony obtained by conventional methods and the DNA- based biosensor. Czech J Food Sci 2003; 21: 78-84.

18. Correia HS, Batista MT, Dinis TCP. The activity of an extract and fraction of *Agrimonia eupatoria* L. against reactive species. Biofactors 2007; 29: 91-104.

19. Gião MS, González-Sanjosé ML, Muniz P, et al. Protection of deoxyribose and DNA from degradation brought about by aqueous extracts of several wild plants. J Sci Food Agric 2008; 88: 633-40.

20. Veterinary laboratory methods: food chemistry (In Slovak). Bratislava: Štátna veterinárna správa Press, 1990: 130-5.

21. Nagy J. Sensory evaluation of poultry and poultry products In: Pribela A, ed. Sensory evaluation of foodstuff, additives and food products (In Slovak). Košice: IVVL Press, 2001: 112-6.

22. Príbela A. Sensory evaluation of foodstuff, additives and food products (In Slovak). Košice: Inštitút vzdelávania veterinárnych lekárov Press, 2001: 191.

23. GraphPad Prism version 4.00 for Windows, GraphPad Software. San Diego, California: GraphPad, 2003.

24. Jang IS, Ko YH, Kang SY, Lee CY. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim Feed Sci Tech 2007; 134: 304-15.

25. Lee KW, Everts H, Beynen AC. Essential oils in broiler nutrition. Int J Poultry Sci 2004; 12: 738-52.

26. Symeon GK, Zintilas C, Demiris N, Bizelis IA, Deligeorgis SG. Effects of oregano essential oil dietary supplementation on the feeding and drinking behaviour as well as the activity of broilers. Int J Poutry Sci 2010; 9: 401-6.

27. Koreleski J, Swiatkiewicz S. Dietary supplementation with plant extract, xantophylls and syntetic antioxidants: effect on fatty acid profile and oxidative stability of frozen stored chicken breast meat. J Anim Feed Sci 2007; 16: 463-71.

28. Nasir Z, Grashorn MA. Effects of *Echinacea purpurea* and *Nigella sativa* supplementation on

broiler performance, carcass and meat quality. J Anim Feed Sci 2010; 19: 94-104.

29. Marcinčák S, Cabadaj R, Popelka P, Šoltýsová L. Antioxidative effect of oregano supplemented to broilers on oxidative stability of poultry meat. Slov Vet Res 2008; 45: 61-6.

30. Marcinčák S, Popelka P, Nagy J, Hussein K, Horňanová Ľ. The impact of vitamin E and rosemary on the oxidative stability of poultry meat and ground meat products. Folia Vet 2004; 48: 165-8.

31. Šperňáková D, Máté D, Rózaňska H, Kováč G. Effects of dietary use of rosemary powder and a-tocopherol on performance of chicken, inhibition of lipid oxidation during storage at chilling conditions and increasing of meat quality. Bull Vet Inst Pulawy 2007; 51: 585-9.

UČINEK FITOGENIH PREHRAMBENIH DODATKOV NA PROIZVODNE PARAMETRE IN KAKOVOSTI MESA PRI BROJLERSKIH PIŠČANCIH

S. Marcinčák, P. Popelka, N. Zdolec, M. Mártonová, J. Šimková, D. Marcinčáková

Povzetek: Cilj poskusa je bil proučiti vplive prahu nageljnovih žbic (*Syzygium aromaticum* L) ter izvlečkov repika (*Agrimonia eupatoria* L) in navadne melise (*Melissa officinalis* L) na proizvodne parameter brojlerskih piščancev, njihove klavne lastnosti in kvaliteto mesa. Pripravljene so bile tri kombinacije dodatkov k prehrani: C – kontrolna skupina brez prehrambenega dodatka in dodatka v prehrani; AC – prehrambeni dodatek s prahom nageljnovih žbic in dodatkom izvlečka repika v pitni vodi; LC – hrana z dodatkom prahu nageljnovih žbic in pitna voda z dodatkom izvlečka melise. Skupina AC brojlerskih piščancev je imela po 14 dneh krmljenja statistično značilno povečano težo ($P < 0.05$) v primerjavi s kontrolno skupino. 42. dan krmljenja je bil pri skupinah brojlerskih piščancev AC in LC opažen boljši izkoristek krme, večji izplen pri klanju ter večja teža prsi in stegen. Statistično značilnih razlik ($P < 0.05$) v izbranih parametrih v poskusnih skupinah AC in LC v primerjavi s kontrolno skupino ni bilo. Suha snov v stegenskih mišicah brojlerskih piščancev skupin AC in LC je bila statistično značilno višja ($P < 0.05$) v primerjavi s kontrolno skupino. V skupinah AC in LC je bila v stegenskih mišicah ugotovljena manjša količina maščobe ($P > 0.05$) kot v kontrolni skupini in statistično značilno ($P < 0.05$) višje razmerje celotnih proteinov v skupini AC. Pri senzoričnem ocenjevanju mesa sta bili skupini AC in LC ocenjeni bolje, čeprav ni bilo opaznih statistično značilnih razlik ($P > 0.05$) v primerjavi s kontrolno skupino.

Ključne besede: repik; nageljnove žbice; rast brojlerskih piščancev; navadna melisa; kakovost mesa

INSTRUCTIONS FOR AUTHORS

Slovenian Veterinary Research contains original articles which have not been published or considered for publication elsewhere. All statements in the articles are the responsibility of the authors. The editorial policy is to publish original research papers, review articles, case reports and abstracts of theses, as well as other items such as critical reviews of articles published in Slov Vet Res, shorter scientific contributions, letters to the editor, etc. Authors should send their contributions to the editorial board's address. All articles are subjected to both editorial review and review by an independent referees selected by the editorial board. The editorial board reserves the right to translate titles, summaries and keywords that have not been translated into Slovene by the authors.

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.

The Summary of 200-300 words should follow on the next page.

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 associated 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.

NAVODILA AVTORJEM

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.

Priloge in slike morajo biti poimenovane z besedami, ki jih opredeljujejo, in arabskimi številkami (npr. Table 1:, Figure 1: itn.). Za dvopičjem sledi besedilo oziroma naslov. Vsi navedki (reference), citirani v besedilu, se morajo nanašati na seznam literature. V besedilu jih je treba oštevilčiti po vrstnem redu, po katerem se pojavljajo, z arabskimi številkami v oklepaju. Prvi navedek v besedilu opredeli številko oziroma vrstni red ustreznega vira v seznamu literature. Če se avtor v besedilu ponovno sklicuje na že uporabljeni vir, navede tisto številko, ki jo je vir dobil pri prvem navedku. Citirana so lahko le dela, ki so tiskana ali kako drugače razmnožena in dostopna javnosti. Neobjavljeni podatki, neobjavljena predavanja, osebna sporočila in podobno naj bodo omenjeni v navedkih ali opombah na koncu tiste strani, kjer so navedeni. V seznamu literature so viri urejeni po vrstnem redu. Če je citirani vir napisalo šest ali manj avtorjev, je treba navesti vse; pri sedmih ali več avtorjih se navedejo prvi trije in doda et al.

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 2011; 48 (1)

Slov Vet Res 2011; 48 (1): 1-34