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Na ovitku: Možni okoljski vplivi proizvodnje krme za molznice, preračunano na tono mleka

(R. Marinšek Logar, I.G. Osojnik Černivec, glej str. 5–26)

On Cover: *Potential environmental impacts of fodder production for dairy cows, calculated per ton of milk*

(R. Marinšek Logar, I.G. Osojnik Černivec, see p. 5–26)

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ENVIRONMENTAL IMPACT EVALUATION OF INNOVATION IN TRADITIONAL FOOD PRODUCTION LINES. PART I: METHODOLOGICAL FRAMEWORK

Ilja Gasan OSOJNIK ČRNIVEC ¹, Romana MARINŠEK-LOGAR ²

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Environmental impact evaluation of innovation in traditional food production lines. Part I: methodological framework

In order to improve the knowledge on environmental impacts of current production systems and to find the solutions to reduce the negative impacts effective multi-approach environmental assessment methodologies are required. The environmental impacts assessed in complex systems, such as agriculture and food production, are prone to higher uncertainties. Therefore, field-specific standardization of the assessment procedures based on multiple screening studies are required to make the assessment outcome less vulnerable. The procedure array pairing for the formation of production step emission inventory is an important methodological process in environmental impact assessment. Initial production data was coupled with specialized databases, models found in scientific literature and environmental category characterization guidelines in order to construct an environmental impact methodological framework specific to four case studies of traditional food production.

Key words: food industry / traditional food products / environmental impact / environmental protection

1 INTRODUCTION

There are many methods that include elements of environmental impact assessment, yet only a few of these allow a multi-approach comprehension of the environmental load.

The set of agri-environmental indicators to be considered ultimately is proposed with great analogy by various governmental and international authorities involved in impact assessment. The European Environ-

Ocenjevanje okoljskih vplivov inovacij v proizvodnji tradicionalnih živil. 1. del: metodološko ogrodje

Analiza okoljskih vplivov dejavnosti agroživilskega sektorja je večplastno opravilo, ki nas pogosto privede do precej negotovih ocen. Oceno vpliva na okolje, v kateri hkrati obravnavamo več vidikov obremenjevanja okolja, lahko izboljšamo s področno-specifično standardizacijo postopkov presoje, ki temelji na podlagi konkretnih primerov iz proizvodnje. Oblikovanje nabora postopkov za pripravo emisijske evidence posameznih proizvodnih korakov je pomemben proces ocenjevanja vplivov na okolje. V našem delu smo eksperimentalne in proizvodne podatke podprli s specializiranimi zbirkami podatkov, modeli iz strokovne literature ter smernicami za karakterizacijo okoljskih kategorij. Predstavljen metodološki okvir smo oblikovali za oceno okoljskega vpliva štirih primerov iz proizvodnje tradicionalnih živil.

Ključne besede: živilska industrija / tradicionalna živila / vplivi na okolje / varstvo okolja

mental Agency (set of agri-environmental indicators under the acronym "IRENA", EEA, 2006), the American Environmental Protection Agency (EPA, 2009), the U.S. Department of Agriculture (Agricultural Resources and Environmental Indicators, USDA, 2006) and the Organization for Economic Cooperation and Development (Environmental Indicators of Agriculture, OECD, 2001) all observe the environmental impact of agriculture from the shared viewpoints of soil, air and water quality, climate change, land consumption, biodiversity, ecosystems

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and human health. More detailed the focus of EEA's agriculture eco-efficiency indicators points to indicators such as energy use, irrigation, emissions of greenhouse gases, acidifying substances and tropospheric ozone precursors.

At the beginning of the environmental impact assessment, the environmental consultant is faced with a set of generic recommendations for the environmental assessment framework elaboration. Therefore, the procedure for the environmental impact assessment prior to the emission inventory construction can vary even for the environmental impact studies of the same specific field as it is dependent upon the expert selection.

Due to the controversy of the procedures applied prior to the emission inventory phase, the methodological frameworks are rarely available or even mentioned in the literature. Nevertheless, it is our opinion, that the pool of the combined data transformation calculations presented beside the environmental impact results of the case studies represents a relevant source for the field specific standardization of environmental impact assessment procedures.

In this part, we present the methodological framework devised for the environmental impact assessment of four case studies (fertility management of *Brassica* sp., dairy cow nutrition, milking frequency and ham cure composition).

2 METHODS

2.1 LIFE CYCLE ASSESSMENT

LCA is a process of evaluating the effects that a product has on the environment over the entire period of its life. It can be used to study the environmental impact of either a product or the function the product is designed to perform. LCA is commonly referred to as a "cradle-to-grave" analysis (EPLC, 2009). Because of their high relevance, good quality and availability of data, the environmental indicators derived from LCA are reported to be effective (Tomassen and De Boer, 2005).

The LCA method is governed by a group of the ISO 14000 environmental management standards (ISO 14040) and is being both promoted and enforced in the European Union (EC-JRC, 2009) and the USA (EPA, 2008), alike.

The LCA study starts with the identification of objectives. After the study system has been described the life cycle inventory (LCI) is developed as a sum of the material and energy flows in and out of the unit process. In the life cycle impact assessment phase (LCIA) of LCA the product system is examined from an environmental

perspective. The inventory results are transferred into contributions to relevant impact categories, such as depletion of abiotic resources, climate change, acidification, etc (ISO 14040).

Acidification (AP) is the air emission of gasses, such as SO_2 , NO_x , HCl and NH_3 . The interaction of these gasses and other molecules of the atmosphere results in the acidification of ecosystems (Audsley *et al.*, 1997). The acidification potential is expressed in equivalents of SO_2 emissions.

Eutrophication (EP) is caused by emission of substrates and gasses (like NO_x , NH_3 , PO_4) to the water and air that affect the ecosystem's growth pattern (De Boer, 2003). The eutrophication potential is expressed in equivalents of PO_4 or NO_3 emission.

Global warming (GHG) refers to the atmospheric greenhouse gasses emissions, which trap some of the reflected outgoing solar energy and retain heat somewhat like the casting of the greenhouse. Still, without this very effect, the Earth's temperature would be lower than it is and life as we know it would not be possible (IPCC, 2001). The greenhouse gas emissions are expressed in equivalents of CO_2 emissions.

Tropospheric ozone precursor potential (TOPP) represents near ground ozone formation as instigator of summer smog. It is the equivalent of ozone formation calculated from ozone precursors (GEMIS, 2009).

Use of **resources** is nowadays treated from the perspective of non-renewable resources. However, efficient use of resources such as fuels, water and agricultural land remains an important topic in agricultural LCAs (De Boer, 2003). The resource consumption is assigned to cumulative energy use (CUE).

An important part of the employed resources is the area of **land** used for the production. This limited resource should not be overlooked in the studies of environmental impact concerning agriculture or forestry studies (Mattsson *et al.*, 2000).

2.2 CASE STUDIES

The procedural principles of LCA were successfully applied to studies of innovative production steps of several traditional food production lines (Table 1). The case studies include the aspects of primary production (vegetable growing – fertilizer treatment, animal husbandry – nutrition and stable practices) and processing of the agricultural products (dry-cured meat) to traditional food products.

The first case study was based on fertilizer treatment field experiments conducted by the Institute for Food and Agricultural Research and Technology (IRTA).

Table 1: Case studies considered in the framework of environmental impact assessment of traditional foods**Preglednica 1:** Študije, vključene v ogrodje ocenjevanja vplivov na okolje v proizvodnji tradicionalnih živil

Process	Case study	Region	Agricultural & production practices	
			traditional	alternative
Fertility management	Vegetable cultivation; <i>Brassica</i> sp.	Spain	mineral fertilizer treatment	organic fertilizer treatment
Feed ration composition	Vegetable oil supplementation; extruded linseed.	France	traditional ration	addition of extruded linseed
Milking frequency	Comparison of once and twice daily milking.	France	twice daily milking	once daily milking
Curing mix manipulation	Reduction of sodium chloride / substitution with potassium lactate.	Spain	high NaCl content in cured ham	reduction of Na ⁺ level

The several field experiments involved the cultivation of different *Brassica* varieties (Trevi, Meridien, Favola), two fertilization treatments (organic fertilizer – manure – O; mineral fertilizer – M) were tested (Alomar *et al.*, 2007; Doltra *et al.*, 2008; Muñoz, 2009).

The second case study was based on nutritional research carried out by the French National Institute for Agricultural Research (INRA). The experiments were focused on the effects of dairy diet supplementation with vegetable oil on the nutritional quality of dairy fat. The

ration fed to cows (Ho-Holstein, Mo-Montbéliarde) was based on different amounts of hay, maize silage, cereal mix, soybean meal and extruded linseeds with and without vitamin E (C-control/traditional, ELS-with extruded linseeds, ELSvE-with extruded linseeds and vitamin E) (Ferlay *et al.*, 2007; Martin *et al.*, 2008).

The third case study was based on a set of milking frequency observations carried out by INRA, likewise. The experiment consisted of three groups (TDM – cows milked twice daily/traditional, ODM – cows milked once

Table 2: Functional units and data characteristics**Preglednica 2:** Funkcionalne enote in glavni viri podatkov

Case study	Functional unit	Main data sources	Assigned to location
Vegetable cultivation; <i>Brassica</i> sp.	t marketable yield ha of land used	Alomar <i>et al.</i> , 2007 Doltra <i>et al.</i> , 2008 Muñoz, 2007 & 2009 MITERRA-EUROPE, 2007 AEMET, 2009 EUSOILS, 2009	Lleida & Tarragona, (Cataluña, Spain)
Vegetable oil supplementation; extruded linseed.	t ECM ^a ha of land used	Ferlay <i>et al.</i> , 2007 Martin <i>et al.</i> , 2008 Martin, 2009 NRC, 2001 MITERRA-EUROPE, 2007 GEMIS 4.5, 2009	Marcenat (Chantal, France)
Comparison of once and twice daily milking.	t ECM ^a ha of land used	Martin <i>et al.</i> , 2007 Pomiès, 2009 NRC, 2001 MITERRA-EUROPE, 2007 GEMIS 4.5, 2009	Monts-Dore (Auvergne, France)
Reduction of sodium chloride / substitution with potassium lactate.	t green ham t cured ham	Fulladosa <i>et al.</i> , 2007 Gou <i>et al.</i> , 2007 Serra <i>et al.</i> , 2007 Arnau, 2007 García, 2009 Fulladosa <i>et al.</i> , 2009 GEMIS 4.5, 2009	Spain

a = energy corrected milk (Sjaunja *et al.*, 1991)

daily, ODMc – cows milked once daily, with calves). The cows were fed *ad libitum* with a pre-mixed ration of grass, maize silage, hay, straw and concentrates (Martin *et al.*, 2007; Pomiès, 2009).

The last case study was based on experiments aimed to reduce the concentration of sodium chloride in dry cured ham, performed by IRTA. Potassium lactate was used as a substitute to Sodium chloride in ham cure (S – traditional NaCl, SR – reduction of NaCl, SRL – NaCl substitution with K-lactate) were tested in the experiment for their effect on weight loss and sensory characteristic (Arnau, 2007; Fulladosa, 2007; Gou, 2007; Serra, 2007).

3 METHODOLOGICAL FRAMEWORK

A well defined scope of the environmental impact assessment is essential. A highly specific LCA, for example, can be employed to discover the points in the production where the potential for environmental burdening is the highest. On the other hand, conceptual LCA can be set on evaluating the potentials of known environmental impact production hot spots (EEA, 1997).

The evident scope of these case studies was to review and assess the environmental impacts of identified production steps. Yet, the underlining goal was the overview of the suitability and development status of the LCA method, the available publicly available tools and data relevant to the agriculture and food production in the EU.

The main function of the selected processes is the production of food. Therefore, the final representation

Table 3: Framework of environmental impact assessment for studied process innovations: GHG, AP and EP categories

Preglednica 3: Konceptualni pregled postopka ocenjevanja okoljskih vplivov, okoljske kategorije: toplogredni plini, potencial za zakisanje in potencial za evtrofikacijo

Process	Category	Emissions	Transformation to emissions	Category characterization
Vegetable oil supplements ^s	GHG	CH ₄ enteric fermentation	– energy intake; NRC, 2001	IPCC, 2006
		CH ₄ from manure	– VS excretion; Fox, 2004	
		N ₂ O from manure	– N excretion; NRC, 2001	
&	AP	NH ₃ to air	– urinary N; Bannink, 1999	EMEP / CORIANIR, 2006
Milking frequency ^s	EP	N leaching	MITERRA-EUROPE, 2007 ^c	Lindfors <i>et al.</i> , 1995 Wenzel, <i>et al.</i> 1998
		N & P runoff		
		from feed production	GEMIS 4.4 & 4.5 & Schmidt <i>et al.</i> , 2004	
Curing mixture manipulation ^s		from Ca production	GEMIS 4.4 & 4.5	Lindfors <i>et al.</i> , 1995 Wenzel <i>et al.</i> , 1998 IPCC, 2006 Brentrup <i>et al.</i> , 2004a & b
		from K production		
		from lactic acid production	Vink <i>et al.</i> , 2007 Vink <i>et al.</i> , 2003	
		from NaCl production	Gonzalez & Overcash, 2000	
Fertility management ^s	GHG	direct N ₂ O from manure	– N applied; EMEP/CORIANIR, 2007	IPCC, 2006
		indirect N ₂ O from manure		
		direct N ₂ O from mineral	– soil organic carbon and sand content; EMEP/CORIANIR, 2007	
	AP	NH ₃ to air	EMEP/CORIANIR, 2007	Wenzel <i>et al.</i> , 1998
	EP	NH ₃ air-water	Brentrup, 2004a & b	Lindfors <i>et al.</i> , 1995 Wenzel, <i>et al.</i> 1998
		NO ₃ leaching	– N balance; Brentrup <i>et al.</i> , 2000	
		N & P runoff	Smith <i>et al.</i> , 2001a & 2001b	
		from fertilizer production	GEMIS 4.4 & 4.5	

c = method in development; s = site specific; g = general

the environmental impacts assessed was expressed per tone of food product and per hectare of agricultural land used for the production (Table 2).

The hot points previously identified were taken as a backbone (objectives identification & LCI construction) for the LCA assessment boundaries (Osojnik Črnivec & Marinšek-Logar, *in press*).

3.1 INVENTORY AND CATEGORY CHARACTERIZATION

The experimental data obtained at INRA and IRTA (TRUEFOOD, 2009) was supported with the expert databases on e.g. fodder composition, nutrition data, weather data, soil characteristics and similar (Table 2). To obtain an emission inventory, initial parameters were coupled with models found in scientific literature. The characterization to categories was performed in accordance with available environmental impact assessment guidelines (Table 3).

Emissions to air (greenhouse & acidification inducing substances) were assessed with the aid of EMEP/CORINAIR Emission Inventory Guidebook (2007) and the IPCC Guidelines for National Greenhouse Gas Inventories (2006).

The preliminary results from a model-in-development, MITERRA-EUROPE, funded under the European Commission, Directorate-General Environment (service contract "Integrated measures in agriculture to reduce ammonia emissions") were used for the localization of emission eutrophication potential in the regions of the experiments.

For the vegetable cultivation case study, the amounts of nutrients in fertilizers and cauliflower marketable yield were taken as the backbone of the environmental impact assessment. Nutrient losses were estimated for NO_3 – leaching (with the aid of N field balance, Brentrup *et al.*, 2000, 2004a & 2004b) and for N and P runoff (Smith *et al.*, 2001a & 2001b).

The NO_3 leaching model required the input of climatic parameters (precipitation), which were obtained for the Cataluña region from the Spanish meteorological agency (AEMET, 2009).

The production of manure is a part of the environmental impact balance of the animal production system and it should be assigned to the animal product produced (milk, meat ...), therefore, the environmental impact of manure production in this case is null.

The production of mineral fertilizers was estimated with the aid of GEMIS 4.5 (2009).

In the case studies of vegetable oil supplementation and milking frequency, the nutritional data was taken as

the basis for environmental impact assessment. In the first stage, the cows' nutritional requirements and the amounts of feed components (indigestible dry matter, crude protein & nitrogen, phosphorus, potassium, sodium ... intake) were estimated (NRC, 2001).

The intake parameters were coupled with existent nutrition-based models (NRC, 2001; Fox, 2004) to assess the levels of excreted nutrients.

The environmental impact of feed production was modelled separately with the GEMIS 4.5 database and tool (2009). For soy meal feed component, production in the Americas and transport to EU was assumed.

Bran wheat (a component of the linseed mixture) is as by-product of the milling process. As it is in excess in the market it serves as a component in animal feed. It has been assumed (LCA Food Database, 2007) that one kg bran displaces one kg of barley.

Straw is a by-product of crop production and is the source of dietary fibre. It has been assumed that 1kg of straw displaces its nutritional equivalent of roughage (approx. 0.9 kg hay).

The production efficiency of dairy cows accounts for milk yield and milk composition. For the standardization purposes, fat corrected milk (Gaines, 1927) – FCM (milk yield usually adjusted to 4 or 3.5% fat) and energy corrected milk (Sjaunja *et al.*, 1991) – ECM (milk yield adjusted to 3.14 MJ/kg; 38.3 MJ fat / kg, 24.2 protein / kg, 4.61% lactose & 0.2% citric acid) are commonly used. For the final expression of the environmental impact, energy corrected milk was calculated with the equation proposed by Sjaunja *et al.* (1991). Hence the composition of the milk in the experiment differed significantly amongst the tested groups; ECM enabled a more relevant comparison of the environmental impacts than the comparison of mere milk yields.

Additionally to the milking frequency experimental groups TDM, ODM and ODM-c, a 'calf-allocation' group, ODMc-a, was created for the calculation and evaluation purposes. In the case of the group ODMc, the full environmental impact cannot be assigned merely to milk produced, as beef meat is the outcome of the calves' production. Therefore in the group ODMc-a, the nutritional requirement of the dairy cows in the ODMc group were subtracted the requirements for milk consumed by calves.

In the potassium lactate production, the production lines of potassium, calcium sulphate and lactic acid are involved (Fig. 1). Lactic acid is produced from sugars by fermentation. Agents, such as calcium sulphate are used, to neutralize the fermentation broth and fixate the product (calcium lactate is formed). For K-lactate production, the calcium ions are replaced via ion exchange with potassium (PURAC, 2009).

GEMIS 4.5 data was used to calculate the environmental impact of calcium sulphate (gyps) and potassium production. As plaster is an input as well as an output of the production (it is recovered in the process), the environmental impact from gyps production was omitted.

The production of lactic acid was extrapolated from data reported by Vink *et al.* (2003) and Vink *et al.* (2007).

The emission inventory of González & Overcash (2000), based on energy sub-modules was used to gain insight into the environmental impact of salt production.

Emissions to air (greenhouse & acidification inducing substances) were assessed with the aid integrated procedures of the GEMIS 4.5 tool and additional characterization factors for greenhouse gas emissions (IPCC, 2007), acidification potential (Wenzel *et al.*, 1998), eutrophication (Lindfors *et al.*, 1995, Wenzel *et al.*, 1998, Brentrup *et al.*, 2004) and photochemical ozone creation potential (EEA, 2000).

4 CONCLUSIONS

The LCA application in the agricultural sector is rapidly accelerated by the need to assess the environmental impact growing of currently controversial energy-crops (Zah *et al.*, 2007) and a need to relevantly define the term “renewable energy”.

The primary production originating from agriculture is commonly recognized as the largest contributor to the environmental impact of food production (Berlin, 2002; Foster *et al.*, 2006, Williams *et al.*, 2006). As the environmental impacts assessed in complex systems are prone to higher uncertainties field – standardization of the assessment procedures based on multiple screening

studies is required to make the assessment outcome less venerable.

The individual array of procedures paired for each environmental impact of the observed production steps is an important result. The study – specific environmental assessment protocols, applied models and databases are suitable for latter preparation of guidelines for environmental impact assessment in specific fields of agriculture and food production.

For a relevant environmental impact several conditions have to be fulfilled. Firstly, the production data of appropriate detail have to be available. Secondly, the direct emission measurements or alternatively the methodology for conversion of the existent production data to the emissions inventory should be available, as it is not the goal of the environmental consultants to develop new assessment models.

During the environmental impact assessment of selected innovations, we encountered several appropriate and well validated models for the evaluation of nutrition-related environmental impact (e.g. NRC, 2001; Fox, 2004). The transfer of nutrients in the environment is sufficiently covered (e.g. EUROHARP, 2004), and the procedures, methods and models in the field of food production are less widespread (e.g. Foster *et al.*, 2006; Roy *et al.*, 2009).

As global warming is an important topic, the methodology for the assessment of GHG emissions is well established. The Intergovernmental Panel on Climate Change (IPCC, 2007) and the European Environmental Agency (EMEP/CORIANIR, 2006) both provide guidelines and workbooks for the assessment of GHG emissions applicable to the field of agriculture and food production, as well.

The assessment of the acidification and eutrophication

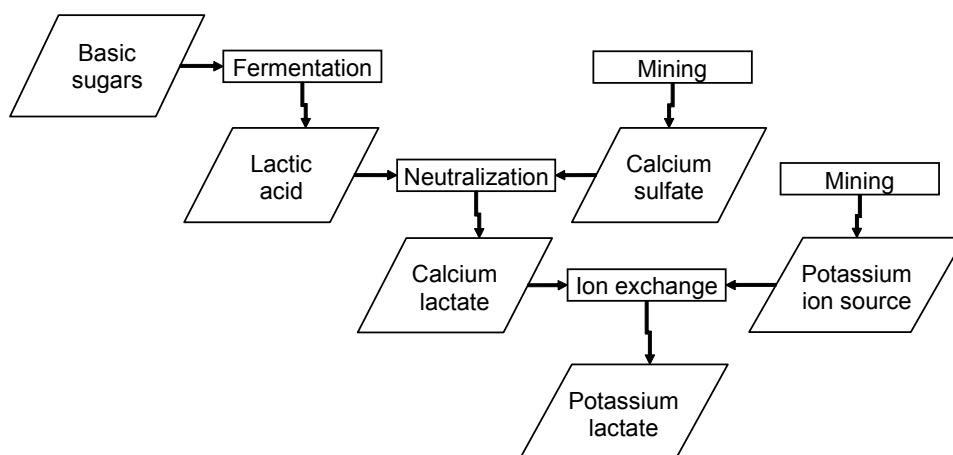


Figure 1: Production of potassium lactate
Slika 1: Proizvodnja kalijevega laktata

cation potential is not yet a unified task. The acidification and eutrophication – related emissions in the dealt cases are specific to the production location. However, general data are available (EUSOILS, 2009), the MITERRA-EUROPE approach is inclining to the local approach (NUTS-2 resolution) and the composition of EU agricultural soils is to be mapped out (EuroGeoSurvey, 2009). The data on land use and energy consumption is not easily available, yet it can be assessed with a relative low-effort when sufficient production data is at hand. The data on photochemical ozone creation potential is commonly scarce in the public domain and should be expected to become more accessible with further development of the global warming assessment methodology.

It is necessary to widen the life cycle inventories to the national detail. Commercial databases dispose with a wide array environmental data for specific processes or products, but (at least for those connected with agriculture) their national / spatial / local relevance is questionable.

It will become necessary to assess a wider array of environmental categories for agricultural activities. Human and ecosystem toxicity, biodiversity depletion and water consumption are relevant environmental issues requiring relevant and publicly available indicators.

The example of the systematic guidelines on GHG emission assessment and/or reporting (EMEP/CORINAIR, 2006; IPCC, 2007) should be taken forward to the construction of national environmental impact inventory guidelines. As different levels of detail are necessary for different assessment types, the procedures should be flexible and allow some different degrees of focus.

The first step towards the conceptual national guideline framework is the summarization of case-specific studies and existent guidelines. Amongst others, the guidelines on LCA tailored to production of crops (Brenttrup *et al.*, 2004a & 2004b), soy meal (Lehuger, 2009) and farming (Thomassen *et al.*, 2009) were taken into consideration whilst performing this environmental impact assessment.

The increasing availability of the tools (such as GEMIS, or the recently developed versatile application, openLCA) and databases (like the European based ELCD, or the NREL LCi database from the US) in the public domain, further support the development of the national and EU guideline formation (e.g. International Reference Life Cycle Data System – ILCD, in development).

5 POVZETEK

V celokupni oceni vpliva proizvodnje živil na okolje nosijo postopki kmetijske pridelave večji delež od vseh

ostalih postopkov procesiranja, razvoza in prodaje (Berlin, 2002; Foster *et al.*, 2006, Williams *et al.*, 2006).

Zaradi raznolikih in pogosto nejasnih postopkov ocenjevanja okoljskih vplivov nas lahko okoljsko vrednotenje kompleksnih proizvodnih sistemov privede do ocen z visoko negotovostjo. Vendarle pa so tudi takšne začetne študije lahko v pomoč pri potrebni standardizaciji področno-specifičnih postopkov presoje vpliva na okolje.

S tem namenom je prvi del našega prispevka namenjen predstavitvi metodološkega ogrodja, ki smo ga uporabili za ocenjevanje okoljskega vpliva, temelječega na metodi ocenjevanja življenjskega cikla. Na podlagi predstavljenega nabora postopkov smo ocenili spremembo okoljskih vplivov pri uvajanju inovacij v proizvodnje linije na štirih primerih (raba gnojil v pridelavi cvetače, sestava obroka krav molznic, pogostnost molže in spreminjanje sestave zorilne mešanice v proizvodnji pršuta).

Eksperimentalne podatke smo pridobili iz inštitutov INRA in IRTA. Prejete podatke smo podkrepili s podatki iz specializiranih baz podatkov (npr. sestava krmil, vremenski podatki, sestava in značilnosti tal itd.). Proizvodne podatke smo pretvorili v emisije s pomočjo ustreznih modelov iz znanstvene literature. Iz popisa emisij smo izračunali doprinos k posameznim kategorijam okoljskih vplivov v skladu z razpoložljivimi smernicami za presojo vplivov na okolje.

Emisije v zrak (toplogredni plini in prekurzorji kislega dežja) smo ocenili s pomočjo vodnika EMEP/CORINAIR (2007) in smernicami IPCC (2006). Za geografsko umestitev ocene evtrofikacijskega potenciala smo uporabili uvodne rezultate projekta MITERRA EUROPE (2009).

V prvi fazi smo pridobljene nabore postopkov povezali individualno za oceno vpliva posameznega proizvodnega koraka na okolje. Ti študijam-specifični protokoli presoje vplivov na okolje in uporabljeni modeli ter baze podatkov so primerni tudi za kasnejšo pripravo smernic za poročanje vplivov na okolje v obravnavanih področjih kmetijske dejavnosti in proizvodnje živil.

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7 REFERENCES

- AEMET – La Agencia Estatal de Meteorología. 2009. <http://www.aemet.es/en/portada> (12. Nov. 2009)
- Alomar O., Arnó J., Gabarra R., Muñoz P., Doltra J., Castelari M. 2007. Interim report on the effect of novel pest control strategies on the incidence of pests, yield and the nutritional quality of fresh lettuce, and of the effect of fertility management systems on the nutritional quality of *Brassica*. 2007. IRTA. A report to the TRUEFOOD project N° D4.3.2-2: 13 p.
- Arnau J. 2007. »The basic characteristics of the Spanish dry cured production line« IRTA (Personal communication, 23. Feb. 2007)
- Audsley E., Alber S., Clift R., Cowell S., Crettaz P., Galiard G., Hasusheer J., Jolliet O., Kleijn R., Mortensen B., Pearce D., Roger E., Teulon H., Weidema B., van Zeijts H. 1997. Harmonization of Environmental Life Cycle Assessment for Agriculture. Final report to the European Commission. European Commission.
- Bannink A., Valk H., Van Vuuren A.M. 1999. Intake and Excretion of Sodium, Potassium and Nitrogen and the Effects on Urine Production by Lactating Dairy Cows. *Journal of Dairy Science*, 82: 1008–1018
- Berlin J. 2002. Environmental life cycle assessment (LCA) of Swedish semi-hard cheese. *International dairy journal*, 12: 939–953
- Brentrup F., Küsters J., Kuhlmann H., Lammel J. 2000. Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector. *International Journal of LCA*, 5: 349–357
- Brentrup F., Küsters J., Kuhlmann H., Lammel J. 2004a. Environmental impact assessment of agricultural production systems using the life cycle assessment methodology: I. Theoretical concept of a LCA method tailored to crop production. *European Journal of Agronomy*, 20, 3: 247–264
- Brentrup F., Küsters J., Lammel J., Barraclough P., Kuhlmann H. 2004b. Environmental impact assessment of agricultural production systems using the life cycle assessment (LCA) methodology II. The application to N fertilizer use in winter wheat production systems. *European Journal of Agronomy*, 20, 3: 265–279
- De Boer I.J.M. 2003. Environmental impact assessment of conventional and organic milk production. *Livestock Production Science*, 80: 69–77
- Doltra J., Ribas A., Alomar O., Gabarra R., Arnó J., Muñoz P., Castelari M., García J.A., Sárraga C. 2008. Interim report on the effect of novel pest control strategies on the incidence of pests, yield and the nutritional quality of fresh lettuce, and of the effect of fertility management systems on the nutritional quality of *Brassica*. IRTA. A report to the TRUEFOOD project N° D4.3.2-3: 18 p.
- EC-JRC (European Commission's Joint Research Centre). 2009. Life Cycle Thinking. <http://lct.jrc.ec.europa.eu/> (27. Oct. 2009)
- EEA (European Environment Agency). 1997. Life Cycle Assessment, A guide to approaches, experiences and information sources. Environmental Issue Series no. 6. <http://reports.eea.europa.eu/GH-07-97-595-EN-C/en> (28. Oct. 2009)
- EEA. 2000. Environmental Signals 2000. European Environmental Agency, Copenhagen, Denmark
- EMEP/CORINAIR. 2006. Emission Inventory Guidebook–2006. <http://reports.eea.europa.eu/EMEP/CORINAIR4> (20. May 2009)
- EMEP/CORINAIR. 2007. Emission Inventory Guidebook–2007. <http://www.eea.europa.eu/publications/EMEP/CORINAIR5/> (05. Nov. 2009)
- EPA. 2009. (U.S. Environmental Protection Agency), Life Cycle Assessment (LCA). <http://www.epa.gov/nrmrl/lcaccess/> (27. Oct. 2009)
- EPLC. 2009. European Platform on Life Cycle Assessment. <http://lca.jrc.ec.europa.eu/> (30. Oct. 2009)
- EuroFIR. 2009. (European Food Information Resource Network), Traditional Foods. <http://www.eurofir.net/public.asp?id=4292> (27. Oct. 2009)
- EuroGeoSurveys. 2007. Association of the Geological Surveys of Europe. <http://www.eurogeosurveys.org/> (20. May 2009)
- EUROHARP. 2004. <http://euroharp.org/index.htm> (20. May 2009)
- EUSOILS – European Soil Portal Home Page. 2009. <http://eusoils.jrc.ec.europa.eu/> (20. May 2009)
- Ferlay A., Chilliard Y., Martin B. 2007. Interim Report on the effect of vegetable oil and/or antioxidant supplementation of dairy diets on the nutritional composition, sensory quality and oxidative stability of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1a-1: 12 p.
- Foster C., Green K., Blenda M., Dewick P., Evans B., Flynn A., Mylan J. 2006. Environmental Impacts of Food Production and Consumption: A report to the Department for Environment, Food and Rural affairs. London. Manchester Business School & DEFRA
- Fox D.G., Tedeschi L.O., Tylutki T.P., Russel J.B., Van Ambrugh M.E., Chase L.E., Pell A.N., Overton T.R. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology*, 112: 29–78
- Fulladosa E., Serra X., Gou P., Arnau J. 2007. Interim report on the effect of potassium lactate and salt contents on the texture and flavour properties. IRTA. A report to the TRUEFOOD project N° D4.2.3-3: 8 p.
- Fulladosa E., Serra X., Gou P., Arnau J. 2009. Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, 82: 213–218
- Gaines W.L. 1927. The Energy Basis of Measuring Milk Yields. *Journal Of Animal Science*: 33–36

- Garcia N. 2009. "Details on deboning-salting-binding" IRTA (personal communication, 16. Feb. 2009)
- GEMIS 4..5 –Global Emission Model for Integrated Systems. 2009. ÖKO Institut e.V.
<http://www.oeko-institut.de/service/gemis/en/index.htm> (28. Oct. 2009)
- González C.J., Overcash M. 2000. Energy sub-modules applied in life-cycle inventory of processes. *Clean Products and Processes*, 2: 57–66
- Gou P., Costa A., Arnau J. 2007. Interim Report on Conjoint Diffusivities of K-lactate and salt at different pH and temperatures. IRTA. A report to the TRUEFOOD project N° D.4.2.3-1: 14 p.
- IPCC. 2001. Climate change 2001. The Scientific Basis. Contribution of the working group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press
- IPCC. 2006 IPCC Guidelines for National Greenhouse Gas Inventories.
<http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html> (12. Nov. 2009)
- IPCC. 2007. Intergovernmental Panel on Climate Change. Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Cambridge University Press
- ISO 14040. 2006. Environmental management -- Life cycle assessment -- Principles and framework
- LCA food database. 2007.
<http://www.lcafood.dk/> (20. May 2009)
- Lehuger S., Gabrielle B., Gagnaire N. 2009. Environmental impact of the substitution of imported soymeal with locally-produced rapeseed meal in dairy cow feed. *Journal of Cleaner Production*, 17: 616–624
- Lindfors L.G., Christiansen K., Hoffmann L., Virtanen Y., Juntilla V., Hanssen O.J., Rønning A., Ekvall T., Finnveden G. 1995. Nordic Guidelines on Life-Cycle Assessment. Nord 20. Copenhagen, Nordic Council of Ministers
- Martin B., Graulet B., Pomiès D. 2007. Interim Report on the effect of once daily milking with and without calves on the nutritional composition of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1c-1: 9 p.
- Martin B., Ferlay A., Cornu A., Chilliard Y. 2008. Final Report on the effect of vegetable oil and/or antioxidant supplementation of dairy diets on the nutritional composition, sensory quality and oxidative stability of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1a-3: 19 p.
- Martin B. 2009. "Meal composition in the oil supplementation experiment" INRA (personal communication, 03. Mar. 2009)
- Mattsson B., Cederberg C., Blix L. 2000. Agricultural land use in life cycle assessment (LCA): case studies of three vegetable oil crops. *Journal of Cleaner Production*, 8–4: 283–292
- MITERRA-EUROPE. 2009. SC ammonia. ALTERRA research institute.
<http://www.scammonia.wur.nl/UK/> (20. May 2009)
- Muñoz P. 2007. "Basic characteristics of cauliflower production in Spain" IRTA (personal communication, 09. Mar. 2007)
- Muñoz P. 2009. "Details on fertilizer treatment in the *Brassica* field experiment" IRTA (personal communication, 16. Mar. 2009)
- NRC – National Research Council. 2001. Nutrient Requirements of Dairy Cattle, seventh revised ed. National Academy Press, Washington, DC, USA
- Osojnik Črničev I.G., Marinšek Logar R. 2009. Identification of Environmental Impact Hot Spots in Traditional Food Production Lines. *Acta Agriculturae Slovenica*, 94, 1: *in press*
- Pieniaka Z., Verbeke W., Vanhonacker F., Guerrero L., Hersleth M. 2009. Association between traditional food consumption and motives for food choice in six European countries, research report. *Appetite*, 53, 1: 101–108
- Pomiès D. 2009. "Meal composition in the milking frequency experiment" (personal communication, 3. Mar. & 8. Apr. 2009)
- PURAC: lactic acid, lactates and gluconates. 2009.
http://www.purac.com/purac_com/ (20. May 2009)
- Roy P., Nei D., Orikasa T., Xu Q., Okadome H., Nakamura N., Shiina T. 2009. A review of life cycle assessment (LCA) on some food products. *Journal of Food Engineering*, 90: 1–10
- Schmidt A.C., Jensen A.A., Clausen A.U., Kampstrup O., Postlethwaite D. 2004. A Comparative Life Cycle Assessment of Building Insulation Products made of Stone Wool, Paper Wool and Flax. *International Journal of LCA*, 9, 1: 53–66
- Serra X., Fulladosa E., Gou P., Arnau J. 2007. Interim report on the effect of novel deboning-salting-binding methodologies. IRTA. A report to the TRUEFOOD project N° D4.2.3-4: 14 p.
- Sibbel A. 2007. The sustainability of functional foods. *Social Science & Medicine*, 64, 3: 554–561
- Sjaunja L.O., Baevre L., Junkkarinen L., Pedersen J., Setälä J. 1991. A Nordic Proposal for an Energy Corrected Milk (ECM) Formula. EAAP Publication No. 50: 156–157
- Smith K.A., Jackson D.R., Pepper T.J. 2001a. Nutrient losses by surface run-off following the application of organic manures to arable land. 1. Nitrogen. *Environmental Pollution*, 112, 1: 41–51
- Smith K.A., Jackson D.R., Withers P.J.A. 2001b. Nutrient losses by surface run-off following the application of organic manures to arable land. 2. Phosphorus. *Environmental Pollution*, 112, 1: 53–60
- Tomassen M.A., De Boer I.J.M. 2005. Evaluation of indicators to assess the environmental impact of dairy production systems. *Agriculture, Ecosystems and Environment*, 111: 185–199
- Tomassen M.A., Dolman M.A., van Calster K.J., de Boer I.J.M. 2009. Relating life cycle assessment indicators to gross value added for Dutch dairy farms. *Ecological Economics* 68, 8–9: 2278–2284
- TRUEFOOD. 2009. (Traditional United Europe Food), Project Results,
<http://www.truefood.eu/page.asp?ID=23> (12. Nov. 2009)
- Vink E.T.H., Rábago K.R., Glassner D.A., Gruber P.R. 2003. Applications of life cycle assessment to NatureWorks™ polylactide (PLA) production. *Polymer Degradation and Stability*, 80: 403–419
- Vink E.T.H., Glassner D.A., Kolstad J.J., Wooley R.J., O'Connor R.P. 2007. The eco-profiles for current and near-future Na-

- tureWorks[®] polylactide (PLA) production. Industrial Biotechnology: 58–81
- Wenzel H., Hauschild M., Alting L. 1998. Environmental assessment of products. Volume2: Scientific background. Springer: 584 p.
- Williams A.G., Audsley E., Sandars D.L. 2006. Determining the environmental burdens and resource use in the production of agricultural and horticultural commodities. Main report, DEFRA research project IS0205. Bedford. Cranfield University & DEFRA
- Zah R., Böni H., Gauch M., Hirsch R., Lehmann M., Wäger P. 2007. Ökobilanz von Energieprodukten: Ökologische Bewertung von Biotreibstoffen. Bern, EMPA: 206 p.

ENVIRONMENTAL IMPACT EVALUATION OF INNOVATION IN TRADITIONAL FOOD PRODUCTION LINES. PART II: CASE STUDIES

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Environmental impact evaluation of innovation in traditional food production lines. Part II: case studies

Case studies of environmental impact assessment were performed for production steps of three traditional food production lines (dairy cow breeding, dry-cured ham, *Brassica* sp. growing) in order to examine the previously constructed methodological framework. The emission inventory was obtained on the basis of initial experimental data. The emissions were aggregated accordingly for the characterization to environmental impact categories. The contribution of studied process steps to several environmental impact categories was assessed (greenhouse gas emission, acidification potential, eutrophication potential, use of resources and use of agricultural land).

Key words: food industry / traditional food products / cheese / dry-cured ham / *Brassica* / environmental impact / environmental protection / Europe

Ocenjevanje okoljskih vplivov inovacij v proizvodnji tradicionalnih živil. 2. del: primeri študij

V tem prispevku predstavljamo končne rezultate ocenjevanja vplivov konkretnih primerov iz proizvodnih linij tradicionalnih živil na okolje. Na primerih uvajanja inovacij v proizvodnje linije v proizvodnji tradicionalnih živil (sir, pršut, cvetača) smo preverili ustreznost vnaprej vzpostavljenega metodološkega ogrodja za ocenjevanje vplivov na okolje. Na podlagi eksperimentalnih podatkov smo opravili popis emisij, iz popisa pa smo izračunali doprinos proizvodnih postopkov k posameznim kategorijam okoljskih vplivov. Glede na dostopnost in kakovost podatkov smo ocenili doprinos različnih postopkov pridelave k več kategorijam obremenjevanja okolja (izpusti toplogrednih plinov, prekursorji kislega dežja, prekomerno kopičenje hranil v okolju, potencial za nastajanje ozonskih lukenj, raba kmetijskih površin in raba drugih virov).

Ključne besede: živilska industrija / tradicionalna živila / sir / pršut / cvetača / *Brassica* / vplivi na okolje / varstvo okolja / Evropa

1 INTRODUCTION

The notions of innovation and tradition are commonly perceived as counterparts. However, this discrepancy fades, when the historical point of view of innovation is considered. Many traditional products, especially traditional food products, were bearing signs of innovation at the time of their initial introduction.

It has been shown that consumers perceive traditional foods with strong regard to their sensory properties (TRUEFOOD, 2009). Furthermore, innovation in traditional processes may be tolerated by the consumers

as long as the food's intrinsic features (physical, chemical, microbiological or organoleptic) are maintained (EuroFIR, 2009). The production of traditional foods is perceived as sustainable and environmentally friendly, as well.

The environmental impact of the traditional food production is associated with various motives for food choice and consumption (Sibbel, 2007; Pieniaka *et al.*, 2009). Therefore, it is important that the environmental influence of the modified production steps in production of traditional food is not overlooked.

In order to improve the knowledge on environmen-

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tal impacts of current production systems and to find the solutions to reduce the negative impacts effective multi-approach environmental assessment methodologies are required. For this purpose, the life cycle assessment (LCA) – like approach was applied to studied changes in the process steps.

The environmental impact hot spots were identified in four model production lines prior to LCA assessment. These traditional production lines were reconstructed to represent European traditional food products in the sectors of dairy, meat, beverages and vegetables (Osojnik Črnivec & Marinšek-Logar, *in press*).

In the following article we report the LCA-wise case studies that were performed for the production steps within vegetable cultivation (fertility management) and within the production lines of hard cooked cheese (dairy cow nutrition and milking frequency) and dry-cured ham (curing mix manipulation).

2 METHODS

2.1 ENVIRONMENTAL IMPACT ASSESSMENT

Life cycle assessment – like approach was applied to studied changes in the process steps, as described in the previous article (Part I).

The experimental data obtained at INRA and IRTA (TRUEFOOD, 2009) was supported with the expert databases on e.g. fodder composition, nutrition data, weather data, soil characteristics and other. As the emission data was not directly available, initial parameters were coupled with models found in scientific literature for the emission inventory completion. The characterization to environmental impact categories was performed in accordance with available environmental impact assessment guidelines.

Emissions to air (greenhouse & acidification induc-

ing substances) were assessed with the aid of EMEP/CORINAIR Emission Inventory Guidebook (2007) and the IPCC Guidelines for National Greenhouse Gas Inventories (2006).

The localization of eutrophication potential related emissions was performed with the use of preliminary results from the MITERRA-EUROPE (2009) database.

The final representation of the environmental impacts assessed was expressed per ton of food product and also per hectare of agricultural land (in the cases where agricultural production of the product was considered).

2.2 CASE STUDIES

The procedural principles of LCA were successfully applied to studies of innovative production steps of several traditional food production lines (Table 1). The case studies include the aspects of primary production (vegetable growing – fertilizer treatment, animal husbandry – nutrition and stable practices) and processing of the agricultural products (dry-cured meat) to traditional food products.

The first case study was based on fertilizer treatment field experiments conducted by the Spanish Institute for Food and Agricultural Research and Technology (IRTA). Several field experiments involved the cultivation of different *Brassica* varieties (Trevi, Meridien, Favola) and two fertilization treatments (organic fertilizer, manure – O; mineral fertilizer – M). While the mineral fertilizer (KH_2PO_4 135.9 kg/ha, NH_4NO_3 148.9 kg/ha, KNO_3 219.2 kg/ha, 20% N-solution 150 l/ha – Fig. 1) was applied according to usual farm practices, the organic treatment (80 t/ha) took place in a single application (Muñoz, 2009). The yields of different varieties differed significantly within and between the fertilizer treatments (approximate marketable yields t/ha: Trevi-O 8, Trevi-M 17,

Table 1: Case studies considered in the framework of environmental impact assessment of traditional foods

Preglednica 1: Študije, vključene v ogrodje ocenjevanja vplivov na okolje v proizvodnji tradicionalnih živil

Process	Case study	Region	Agricultural & production practices	
			traditional	alternative
Fertility management	Vegetable cultivation; <i>Brassica</i> sp.	Spain	mineral fertilizer treatment	organic fertilizer treatment
Feed ration composition	Vegetable oil supplementation; extruded linseed.	France	traditional ration	addition of extruded linseed
Milking frequency	Comparison of once and twice daily milking.	France	twice daily milking	once daily milking
Curing mix manipulation	Reduction of sodium chloride / substitution with potassium lactate.	Spain	high NaCl content in cured ham	reduction of Na^+ level

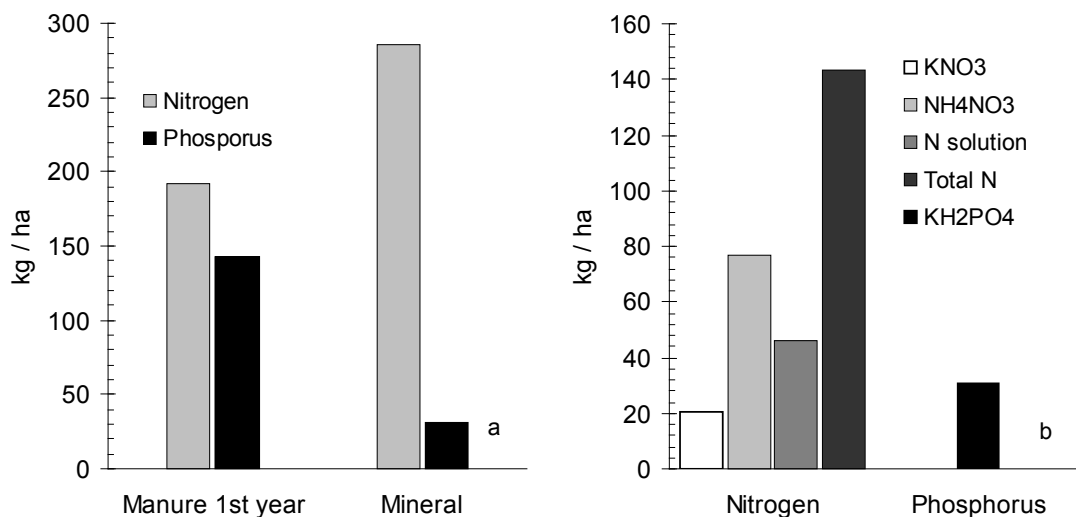


Figure 1: N and P sources in the *Brassica sp.* field experiment (a – by type of fertilizer, b – by mineral fertilizer nutrient sources).
Slika 1: Viri N in P v poljskem poskusu gojenja *Brassica sp.* (a – po vrsti gnojila, b – po vrsti mineralnega gnojila).

Meridien-O 10, Meridien-M 25, Favola-O 29, Favola-M 30).

The amount of nitrogen applied to the field with manure was high (app. 450 kg/ha), but the amount of N available to plants in the short-range is much lower (results for N available in the first year are presented in Fig. 1), thus the N utilized by plants / emitted to the environment is a part of the environmental impact balance of the forthcoming crops. Also, more phosphorus is applied by organic fertilizers than mineral, which can be problematic (due to nutrient loss) especially in P-rich soils.

Further details about the experiment are described in the research reports (Alomar *et al.*, 2007; Doltra *et al.*, 2008).

The second case study was based on nutritional research carried out by the French National Institute for Agricultural Research (INRA). The experiments were focused on the effects of dairy diet supplementation with vegetable oil on the nutritional quality of dairy fat. The ration fed to cows was based on different amounts of hay, maize silage, cereal mix, soybean meal and extruded linseeds (with and without vitamin E). The composition of feed grouped by feeding regimes (C-control/traditional,

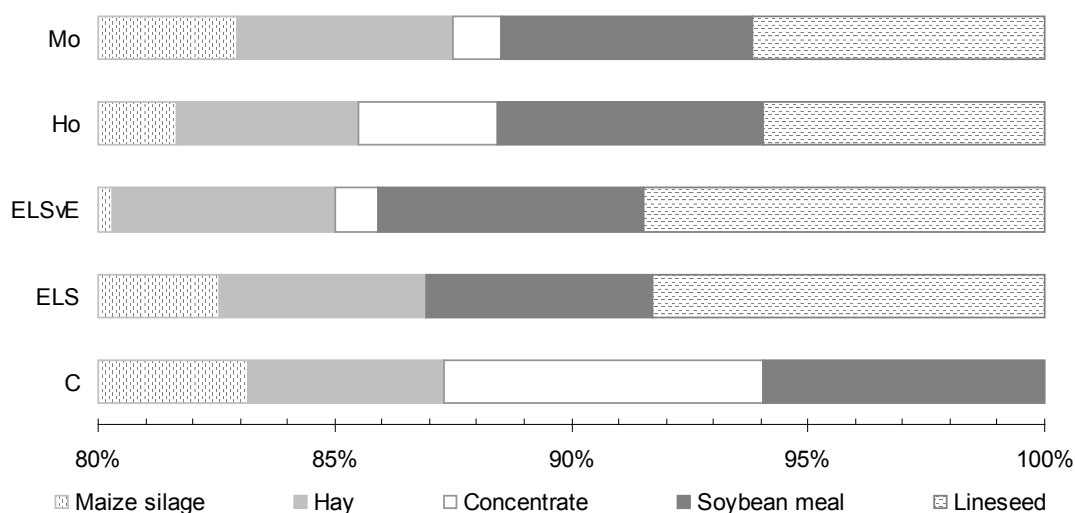


Figure 2: Feeding ration composition (share of consumed fresh matter).
Slika 2: Sestava krmnih obrokov (delež zaužite krme).

Table 2: Contribution to environmental impact categories by fertilizer treatment
Preglednica 2: Doprinos h kategorijam vpliva na okolje po vrsti gnojila

Variety	Treatment	GHG [eq. kg CO ₂]		AP [eq. kg SO ₂]		EP [eq. kg NO ₃] [eq. kg PO ₄]				LU [ha]
		/t	/ha	/t	/ha	/t	/ha	/t	/ha	/t
Trevi	Organic*	230	1870	3.3	26.5	22.9	183	4.4	35.3	0.125
	Mineral	1230	20500	1.04	17.3	0.14	2.3	0.15	2.5	0.060
Meridien	Organic *	190	1870	2.6	26.5	4.5	44	2.2	22.1	0.100
	Mineral	810	20500	0.68	17.3	0.09	2.3	0.10	2.5	0.040
Favola	Organic *	60	1870	0.9	26.5	0.5	13	0.6	19.2	0.034
	Mineral	680	20500	0.58	17.3	0.08	2.3	0.08	2.5	0.033

*= emissions in the first year after application

ELS-with extruded linseeds, ELSvE-with extruded linseeds and vitamin E) or by breeds (Ho-Holstein, Mo-Montbéliarde) in the experiment is presented in Fig. 2. The first results showed no change in the dry matter intake, the milk yield and fat content. The nutritional composition of milk fat was improved, yet the animal weight and the milk protein content were depressed. Further details about the experiments are described in the research reports (Ferlay *et al.*, 2007; Martin *et al.*, 2008).

The third case study was based on a set of milking frequency observations carried out by INRA, likewise. The experiment consisted of three groups (TDM – cows milked twice daily/traditional, ODM – cows milked once daily, ODMc – cows milked once daily, with calves). The nutritional requirement of the dairy cows in the ODMc group were subtracted the requirements for milk consumed by calves to create an additional 'calf-allocation' group, ODMc-a, for the calculation purposes. The cows were fed *ad libitum* with a pre-mixed ration of grass (20% on DM) and maize silage (25% on DM), hay (9% on DM), straw (3% on DM) and concentrates (3% DM on soy meal, 40% on DM on concentrate) (Pomiès, 2009). The modification of the nutritional quality of milk and the decrease of milk yield was demonstrated with the reduction of milking frequencies. Cows milked twice daily had an average yield of 33.66 (32.88 ECM) kg/d, the cows milked once daily with calves 14.68 (14.73 ECM) kg/d and without calves 21.90 (22.87 ECM) kg/d. Further details about the experiments are described in the research report (Martin *et al.*, 2007).

The last case study was based on experiments aimed to reduce the concentration of sodium chloride in dry cured ham, performed by IRTA. Potassium lactate was efficiently used as a substitute to Sodium chloride in ham cure. A salt reduction curing mixture (SR – 15 g NaCl / kg green ham) and salt reduction treatment with added K-lactate (SRL – 15g/kg NaCl, 39.7 g K-lactate / kg green

ham) were tested in the experiment for their effect on weight loss and sensory characteristic. The traditional curing mixture (S), as described by Arnau (2007), contains 30g/kg green ham NaCl. Further details about the experiments are described in the research reports (Ful-ladosa, 2007; Gou, 2007; Serra, 2007).

3 RESULTS AND DISCUSSION

3.1 FERTILIZER TREATMENT

As the global warming potential and acidification potential were based on gaseous emissions from the field (usually prior to planting), these environmental categories are influenced in equal shares when expressed per area utilized and are lower for manure than mineral of agricultural utilization (Table 2). Expressed per ton of yield, GHG and AP expressed per ton of yield, the GHG and AP emissions are the lowest for the highest yield (Favola-O).

The environmental impact of fertilizer treatment is governed by excessive soluble nutrients and most of these nutrients appear to remain unexploited during manure treatment. This is evident especially in the case of Trevi variety, presumably due to the low yield and short time of growth.

Fertilizer production contributes less than 5% of GHG emissions (Fig. 3), more than 20% of AP (Fig. 4) and near 50% of EP (Fig. 5; due to low amount of NO₃ leaching) to the total environmental impact of mineral fertilizers.

According to EMEP/CORINAIR methodology, mineral fertilizer GHG emissions of mineral fertilizer application constitute of direct emissions of N₂O and additional indirect N₂O emissions for manure management (Fig. 3).

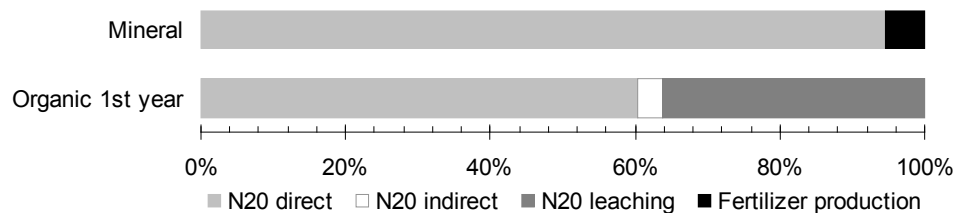


Figure 3: Greenhouse gas emission (GHG) sources of fertilizer treatment.

Slika 3: Viri emisij toplogrednih plinov v povezavi z rabo gnojil.

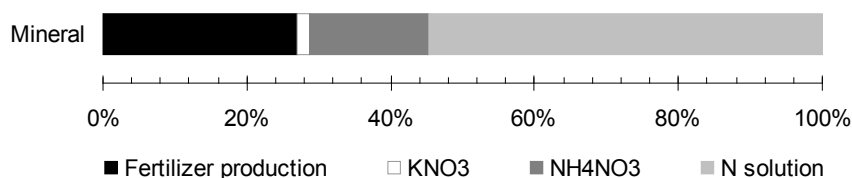


Figure 4: Acidification potential (AP) sources of mineral fertilizer.

Slika 4: Emisijski viri kislega dežja v povezavi z rabo mineralnih gnojil.

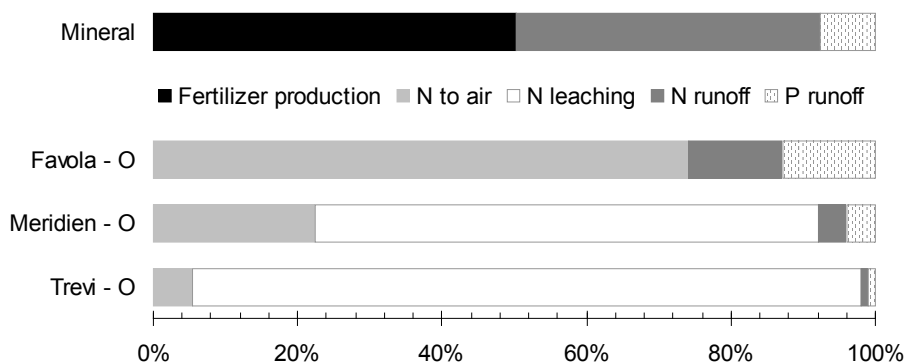


Figure 5: Eutrophication potential (EP) sources of fertilizer treatment.

Slika 5: Emisijski viri prekomernega kopičenja hranil v okolju v povezavi z rabo gnojil.

The acidification potential in this assessment is based entirely on ammonia gaseous emissions. The main contributor to these emissions for mineral fertilizer treatment is the application of N-solution (Fig. 4).

Nitrogen leaching, contributing to the EP, was assessed only in the cases of Meridien and Trevi varieties fertilized with manure (Fig. 5). In other cases, the assessed N-plant-uptake was exceeded or equal to the N-input. These results are quite consistent with the findings of Brenttrup *et al.* (2004a) wheat production studies, which stated that at N rates higher than 144 kg N/ha, the eutrophication potential is dominated by NO_3 leaching.

The amount of gaseous loss of nitrogen is greatest during the denitrification and nitrification of mineral fer-

tilizers (N_2O to air) and direct NH_3 emissions for manure application. The reduction of ammonia field volatilization with the incorporation within some hours after application is possible in the 10-fold range. However, practices that reduce the nutrient losses in one agricultural phase are increasing the input the next stage. To prevent the increase of emissions during growth, i.e. 'pollution swapping', the further steps should be optimized, too.

The ammonia emission from manure is governed by the type of manure (originating species and composition), type of soil, application regime, weather conditions (wind) and so on. Basing on the data provided by Smith *et al.* (2001a), we compared the application of farmyard manure (N input based on experimental conditions)

Table 3: Contribution to environmental impact categories of fodder production by feeding regime and breed
Preglednica 3: Doprinos h kategorijam vpliva proizvodnje krmil na okolje glede na krmni obrok in pasmo

Group	GHG [eq. kg CO ₂]		AP [eq. kg SO ₂]		EP [eq. kg PO ₄] [eq. kg NO ₃]				POCP [eq. kg TOPP]		RU [MJ CEU]	
	/t	/ha	/t	/ha	/t	/ha	/t	/ha	/t	/ha	/t	/ha
C	180	2 290	1.20	15.6	0.035	0.45	0.36	4.6	0.71	9.2	1 840	23 830
ELS	180	1 550	1.18	10.2	0.083	0.72	0.88	7.5	0.43	3.7	3 990	34 340
ELSvE	190	1 600	1.29	10.7	0.088	0.73	0.93	7.6	0.46	3.8	4 220	34 820
Ho	180	1 740	1.22	11.6	0.071	0.67	0.74	7.0	0.51	4.9	3 430	32 790
Mo	190	1 680	1.28	11.2	0.077	0.68	0.81	7.1	0.53	4.6	3 730	32 820

with the autumn application of slurry. The N-runoff from farmyard manure was lower (higher DM and lower urea content) in the cumulative N-runoff. The assessed runoff of NO₃ after application is similar for the studied organic fertilizers, yet the runoff ammonia emissions of farmyard manure are superseded by the surface draining of slurry ammonia. Also, the runoff of NO₃ dominates the total N-runoff in the applied mineral fertilizer array (results not shown).

The traditional mineral fertilizer treatment in this experiment conveys higher GHG emissions and lower AP, EP and LU values than manure application.

The application of manure was most environmentally efficient in the Favola-O scenario, where the marketable yield of cauliflower had least declined comparing to the yield of Favola fertilized with mineral fertilizers.

3.2 VEGETABLE OIL SUPPLEMENTATION

The global warming potential of the »traditional« diet production (group C) was amongst the lowest expressed per fodder consumed in one day or fodder consumed per ton ECM. However, per ha, the level of GHG emissions was noticeably higher than in other groups (Table 3). This is presumably due to cereal mix (group C)

substitution with linseed (Fig. 2). The same relation can be observed for the acidification (AP) and photochemical ozone creation potential (POCP). In cases of eutrophication potentials (EP), land use (LU) and resources use (RU), group C remains most efficient.

The production of feed consumed for the production of 1 ton ECM required 0.0774 ha land in group C, 0.1160 ha in group ELS, 0.1210 ha in group ELSvE. The amount of feed consummated by the dairy cows to produce 1 ton of ECM was produced on 0.1050 ha land for Holstein and 0.1140 ha for the Montbéliarde breed.

The supplementation of linseed increases the resources used for feed production, the eutrophication potential and the use of land, as this crop has a much smaller yield than maize and barley used in the cereal mix.

The Montbéliarde breed appears to have a lower capability of feed conversion and therefore bares higher environmental impacts per ton ECM yield than the Holstein breed.

The majority of the environmental impacts arise from the animal breeding phase (on average 71.8% of total GHG, 92.9% of AP and 98.8% of EP).

The manure management accounts for 30% and enteric fermentation for 70% of the greenhouse gas emissions in animal production. Under the determined conditions, more than half emissions of ammonia con-

Table 4: Contribution to environmental impact categories of animal breeding by feeding regime and breed
Preglednica 4: Doprinos h kategorijam vpliva reje živali na okolje glede na krmni obrok in pasmo

Group	GHG [eq. kg CO ₂]		AP [eq. kg SO ₂]		EP [eq. kg PO ₄] [eq. kg NO ₃]				LU [ha]
	/t	/ha	/t	/ha	/t	/ha	/t	/ha	
C	500	2 846 000	17.5	992 600	5.8	332 200	61.6	3 498 000	0.21
ELS	440	2 576 000	14.7	862 700	5.4	314 600	56.5	3 311 000	0.20
ELSvE	470	2 576 000	16.3	902 200	5.9	325 000	61.9	3 421 000	0.22
Ho	460	2 928 000	15.4	992 800	5.5	354 300	57.9	3 729 000	0.19
Mo	490	2 425 000	17.3	857 300	6.1	301 500	64.0	3 173 000	0.24

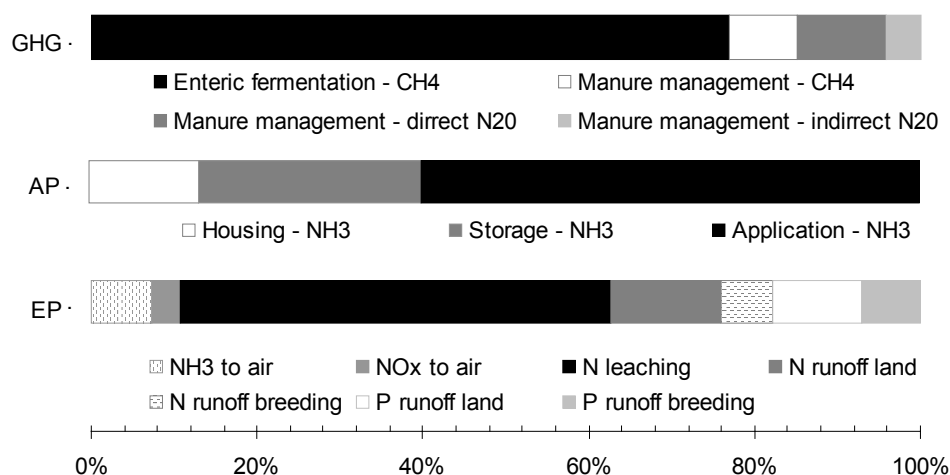


Figure 6: Major sources of environmental impacts in animal husbandry.

Slika 6: Večji viri vpliva reje živali na okolje.

tributing to the acidification potential occur after application of manures to agricultural areas, a third during storage and the least is volatilized in the stable (Fig. 6).

The overall assessment is favourable towards the vegetable oil supplementation from the viewpoint of GHG emissions and AP (10% and 11% average reduction during animal production, respectively).

On the other hand, linseed production requires more energy resources and similar EP and LU than the utilization of the traditional diet. This can be compensated with the use of renewable energy resources and with the purchase of locally produced linseed (for energy), breed selection, nutrition balancing and optimization of agricultural practices (for eutrophication potential) to reduce the environmental impact budget of the innovative production step.

3.3 ONCE DAILY MILKING

As the TDM group produced the most milk and consumed the most fodder, their absolute environmental

impact (per day) was the highest. Expressed per production of milk, the environmental impact for production of feed consumed in the TDM group is the lowest (Table 5). The grounds are nutritional as the cows that produce less milk have more or less the same amount of nutrient requirements for maintenance than cows with a higher amount of milk. When the milk yield decreases the feed consumed for maintenance purposes becomes more evident. The high environmental impact levels of the ODMc group are biased. The bias is revealed by assigning the difference to rearing calves (ODMc-a).

Whilst the cow-ration was pre-mixed and fed *ad libitum* the composition of the feed is uniform. Therefore, the EI expressed per agricultural area used to produce the feed does not vary amongst different test groups and is as follows; 1803.9 eq. kg CO₂ / ha (GHG), 10.70 eq. kg SO₂ / ha (AP), 0.38 eq. kg PO₄ / ha & 3.96 eq. kg NO₃ / ha (EP), 6.58 eq. kg TOPP / ha (POCP), 15872.71 MJ CEU / ha (RU).

The environmental impact of individual feed component is disproportionate to its consumed share and is not uniform amongst the environmental categories (Ta-

Table 5: Contribution to environmental impact of fodder production by milking regime per t ECM

Preglednica 5: Doprinos h kategorijam vpliva proizvodnje krmil na okolje glede na pogostost molže na t ECM

Group	GHG [eq. kg CO ₂]	AP [eq. kg SO ₂]	EP [eq. kg PO ₄]	[eq. kg NO ₃]	POCP [eq. kg TOPP]	RU [MJ CEU]	LU [ha]
TDM	180	1.0	0.037	0.39	0.64	1550	0.97
ODM	240	1.4	0.050	0.52	0.86	2080	0.13
ODMc	390	2.3	0.083	0.86	1.42	3430	0.22
ODMc-a	300	1.8	0.063	0.65	1.08	2600	0.16

Table 6: Contribution to environmental impact categories of animal breeding by milking regime**Preglednica 6:** Doprinos h kategorijam vpliva reje živali na okolje glede na pogostnost molže

Group	GHG [eq. kg CO ₂]		AP [eq. kg SO ₂]		EP [eq. kg PO ₄]		[eq. kg NO ₃]		LU [ha]
	/t	/ha	/t	/ha	/t	/ha	/t	/ha	/t
TDM	460	29960 000	13.3	863 600	4.7	303 300	49.3	3 193 000	0.15
ODM	630	27700 000	19.1	845 000	6.6	291 200	69.4	3 066 000	0.27
ODMc	1040	29230 000	32.9	924 100	11.2	315 200	118.1	3 318 000	0.43
ODMc-a	790	22 160 000	25.2	708 400	8.5	240 000	89.9	2 526 000	0.43

ble 5). The concentrate production carries the bulk of impacts. The impact of soybean is also excessive, yet it is not immediately noticeable since the amount of soy consumed is very small.

Greater daily emissions arise from the agricultural animal breeding practices (on average 72.6% of total GHG, 93.2% of AP and 99.2% of EP).

Similarly to diet production, the environmental impact of the least productive group is the highest expressed per yield of milk (increase of GHG 77%, AP 193%, EP 88%, LU 103%; average % of environmental impact change comparing to traditional TDM) whereas per ha of agricultural area, the levels of environmental impact are equalized or inverted amongst the groups (reduction of GHG 12%, AP 4%, EP 7%; average % of EI change comparing to traditional TDM). This relationship (Table 6) is in accordance to the findings of De Boer (2003) whilst comparing the environmental impact of conventional and organic dairy farming.

Therefore, the measures for environmental impact reduction to compensate for the higher environmental impact of the once daily milking frequency (per milk amount) should be focused on altering the production

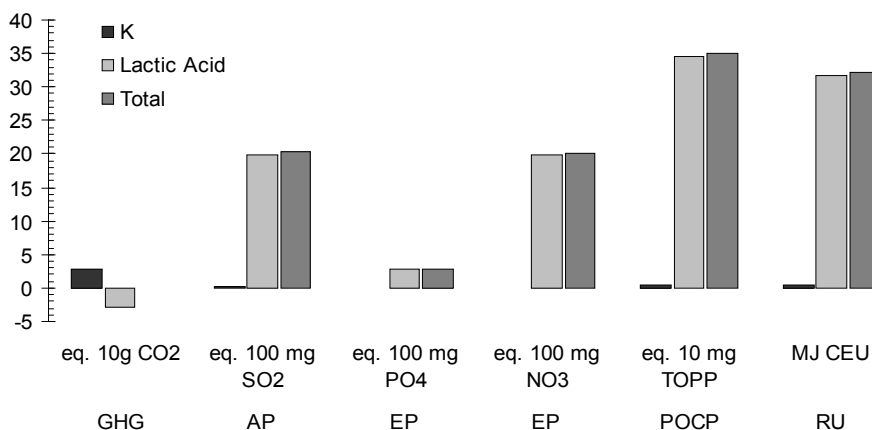
practices related to nutrition, animal housing and manure.

3.4 ADDITION OF POTASSIUM LACTATE

The environmental impact of lactic acid dominates the EI of the potassium lactate production in all the environmental categories (Fig. 7), since considerable amounts of resources are used in the cultivation and fermentation of sugars. However, as sugars are acquired from plant biomass and renewable energy can be used, the GHG potential of lactic acid production is negative (Vink *et al.*, 2003; Vink *et al.*, 2007).

The environmental impacts related to production of potassium lactate are very low (negative for GHG, some 10⁴ ranges greater for AP, EP and POCP and approximately 10 times more energy consuming) comparing to the production of the same amount of NaCl (Fig. 8).

The change in the weight loss does not affect the environmental impact of different cure mixture in the sense of changing the environmental impact relationship levels (Table 7). The traditional cure mix (S) has the highest

**Figure 7:** Environmental impact of potassium lactate component production (/ kg potassium lactate).**Slika 7:** Vpliv proizvodnje komponent kalijevega laktata na okolje (/kg kalijevega laktata).

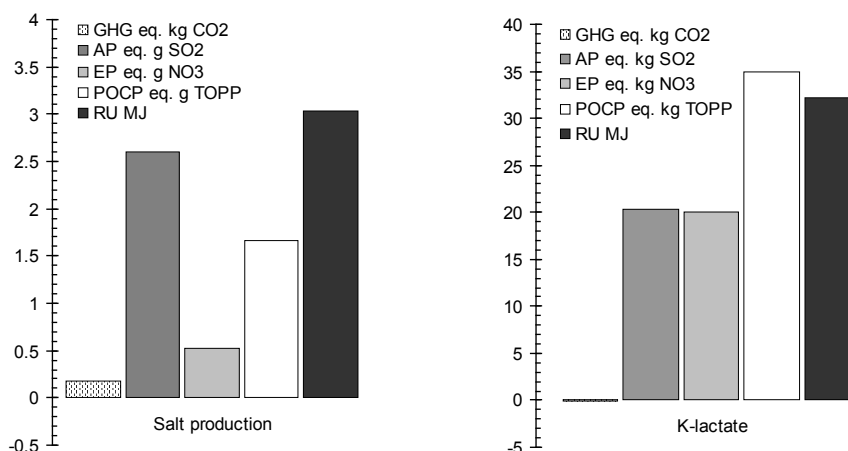


Figure 8: Comparison of the environmental impact of NaCl and K-lactate production (note – different scales are used in the graphs).
Slika 8: Primerjava vplivov proizvodnje NaCl in K-laktata na okolje (pozor – primerjavi sta prikazani na različnih merilnih skalah).

global warming potential, and has higher environmental impact levels than the reduced salt cure (SR) and lower levels than SRL for all others environmental impact categories. The higher amount of energy and resources used for the production of K-lactate (Fig. 8) is evident also in the empirical conditions (Table 7). Being the global warming potential of K-lactate production low (Fig. 7), the GHG emissions of SLR are similar to SR values (Table 7).

Therefore, the SR technique is the most acceptable from the viewpoint of environmental impacts. Environmentally more suitable curing mixture, containing K-lactate, requires further reductions of other curing components. However, an entirely equivalent environmental impact to the traditional cure mix could be difficult to achieve.

4 CONCLUSIONS

The individual arrays of procedures paired to meet the demand of study-specific environmental assessment were successfully applied to production steps studied.

In the *Brassica* sp. field tests, the traditional mineral fertilizer treatment exhibited higher GHG emissions and lower AP, EP and LU values than manure application. The application of manure was most environmentally efficient for the Favola high-yield variety. However, in our opinion, it is important to consider the environmental impacts of other fertilizer treatments (e.g. the combination of mineral and organic fertilizers, anaerobically treated organic manures, green manures ...).

As linseed has a much smaller yield than maize and barley used in the cereal mix, the supplementation of linseed increases the resources used for feed production, the eutrophication potential and the use of land. Yet, the overall assessment is favourable towards the vegetable oil supplementation from the GHG emission and AP point of view. Should the data be available, it is important to consider the flax variety grown for the supplementation (intended only for seed / or also for the production of fibre).

The milking once a day appears to be environmentally friendly when the values per agricultural land used or daily values are observed. Nevertheless, the higher environmental profile of this group is revealed, when the

Table 7: Contribution to environmental impact categories of cure mixture manipulation
Preglednica 8: Doprinos h kategorijam vpliva spreminjanja sestave zorilne mešanice

Group	GHG [eq. kg CO ₂]		AP [eq. kg SO ₂]		EP [eq. kg PO ₄]		[eq. kg NO ₃]		RU [MJ]	
	/t green ham	/t cured ham	/t green ham	/t cured ham	/t green ham	/t cured ham	/t green ham	/t cured ham	/t green ham	/t cured ham
S	5.5	8.4	0.078	0.118	0.00100	0.00152	0.0158	0.024	0.050	0.075
SR	2.8	4.0	0.039	0.057	0.00050	0.00073	0.0079	0.012	0.025	0.036
SRL	2.8	4.1	0.087	0.129	0.00727	0.01078	0.0553	0.082	0.108	0.159

environmental impact is expressed per milk amount. Once the milk ingredients have been standardized with the energy corrected milk equation the increase of the ingredients due to the decrease of the milking frequency does not compensate for the higher environmental impact linked with animal breeding.

The sodium chloride reduction cure was the most efficient, environmental impact wise. The production of potassium lactate employs several material and energy resources. Therefore, an entirely equivalent environmental impact to the traditional cure mix could be difficult to achieve with the cure containing potassium lactate. Nevertheless, the dietetic effects of the potassium lactate could prove to be an important advantage for the use in food products.

POVZETEK

V prvi fazi smo na primerih uvajanja inovacij v proizvodnje linije v proizvodnji tradicionalnih živil (sir, pršut, cvetača) uredili metodološko ogrodje za ocenjevanje vplivov na okolje. Nadalje smo preverili ustreznost pristopa in na podlagi predhodno vzpostavljenega metodološkega ogrodja ocenili vplive posameznih proizvodnih korakov obravnavanih primerov na okolje.

Ti, za študije-specifični protokoli presoje vplivov na okolje in uporabljeni modeli ter baze podatkov, so primerni tudi za kasnejšo pripravo smernic in za poročanje o vplivih na okolje na obravnavanih področjih kmetijske dejavnosti in proizvodnje živil.

Končne ocene okoljskega vpliva smo obravnavali z vidika količine živil (/t izdelka) in z vidika rabe kmetijskih zemljišč (/ha, v tistih primerih, v katerih smo upoštevali kmetijsko proizvodnjo).

V poljskem poskus gojenja cvetače ima raba mineralnih gnojil večji potencial za globalno segrevanje ter nižje potenciale za tvorbo kislega dežja ter prekomernega kopičenja hranil. V splošnem je bila raba gnojil okoljsko najbolj učinkovita za varieteto Favola z visokim hektarskim donosom.

Dokrmeljevanje ekstrudiranega lanenega semena se v proizvodnji krme kaže na povečani rabi naravnih virov, kmetijskih površin in povečanem potencialu za prekomerno kopičenje hranil. Lan ima namreč precej manjši hektarski donos kot koruza in ječmen, ki sta bila sicer uporabljena v koncentratu. Celokupno je dokrmeljevanje lanenega senu upravičeno z vidika toplogrednih emisij in potenciala za tvorbo kislega dežja.

Na prvi pogled molža enkrat na dan okolje v obravnavanih kategorijah obremenjuje v manjši meri kot molža dvakrat na dan. Zaradi skorajda enakih vzdrževalnih potreb krav molznic v obeh eksperimentalnih skupinah,

pa so ocene vpliva pogostnosti molže na okolje mnogo nižje pri živalih, ki so jih molzli dvakrat dnevno.

Z okoljskega vidika je najbolj ugodna sestava zorilne mešanice z manjšo količino natrijevega klorida, kot je običajna v tradicionalnih mešanicah in brez dodatka kalijevega laktata. V proizvodnji kalijevega laktata je poraba materialnih in energetskih virov precej večja kot v pridelavi soli. Z vsakršnim dodatkom kalijevega laktata je zato težko doseči popolni okoljski ekvivalent mešanicam z vsebnostjo kuhinjske soli. Toda v končni fazi vrednotenja ugodnih in neugodnih učinkov te proizvodnje linije ne smemo zanemariti dietetičnih učinkov kalijevega laktata.

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REFERENCES

- AEMET – La Agencia Estatal de Meteorología. 2009. <http://www.aemet.es/en/portada> (12. Nov. 2009)
- Alomar O., Arnó J., Gabarra R., Muñoz P., Doltra J., Castelari M. 2007. Interim report on the effect of novel pest control strategies on the incidence of pests, yield and the nutritional quality of fresh lettuce, and of the effect of fertility management systems on the nutritional quality of *Brassica*. 2007. IRTA. A report to the TRUEFOOD project N° D4.3.2-2: 13 p.
- Arnau J. 2007. »The basic characteristics of the Spanish dry cured production line« IRTA (Personal communication, 23. Feb. 2007)
- Audsley E., Alber S., Clift R., Cowell S., Crettaz P., Galiard G., Hasusheer J., Joliet O., Kleijn R., Mortensen B., Pearce D., Roger E., Teulon H., Weidema B., van Zeijts H. 1997. Harmonization of Environmental Life Cycle Assessment for Agriculture. Final report to the European Commission. European Commission.
- Bannink A., Valk H., Van Vuuren A.M. 1999. Intake and Ex-

- cretion of Sodium, Potassium and Nitrogen and the Effects on Urine Production by Lactating Dairy Cows. *Journal of Dairy Science*, 82: 1008–1018
- Berlin J. 2002. Environmental life cycle assessment (LCA) of Swedish semi-hard cheese. *International dairy journal*, 12: 939–953
- Brentrup F., Küsters J., Kuhlmann H., Lammel J. 2000. Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector. *International Journal of LCA*, 5: 349–357
- Brentrup F., Küsters J., Kuhlmann H., Lammel J. 2004a. Environmental impact assessment of agricultural production systems using the life cycle assessment methodology: I. Theoretical concept of a LCA method tailored to crop production. *European Journal of Agronomy*, 20, 3: 247–264
- Brentrup F., Küsters J., Lammel J., Barraclough P., Kuhlmann H. 2004b. Environmental impact assessment of agricultural production systems using the life cycle assessment (LCA) methodology II. The application to N fertilizer use in winter wheat production systems. *European Journal of Agronomy*, 20, 3: 265–279
- De Boer I.J.M. 2003. Environmental impact assessment of conventional and organic milk production. *Livestock Production Science*, 80: 69–77
- Doltra J., Ribas A., Alomar O., Gabarra R., Arnó J., Muñoz P., Castelari M., García J.A., Sárraga C. 2008. Interim report on the effect of novel pest control strategies on the incidence of pests, yield and the nutritional quality of fresh lettuce, and of the effect of fertility management systems on the nutritional quality of *Brassica*. IRTA. A report to the TRUEFOOD project N° D4.3.2-3: 18 p.
- EC-JRC (European Commission's Joint Research Centre). 2009. Life Cycle Thinking. <http://lct.jrc.ec.europa.eu/> (27. Oct. 2009)
- EEA (European Environment Agency). 1997. Life Cycle Assessment, A guide to approaches, experiences and information sources. Environmental Issue Series no. 6. <http://reports.eea.europa.eu/GH-07-97-595-EN-C/en> (28. Oct. 2009)
- EEA. 2000. Environmental Signals 2000. European Environmental Agency, Copenhagen, Denmark
- EEA. 2006. Integration of environment into EU agriculture policy – the IRENA indicator-based assessment report. http://reports.eea.europa.eu/eea_report_2006_2/en/IRENA-assess-final-web-060306.pdf (29. Avg. 2007)
- EMEP/CORINAIR. 2006. Emission Inventory Guidebook–2006. <http://reports.eea.europa.eu/EMEP/CORINAIR4> (20. May 2009)
- EMEP/CORINAIR. 2007. Emission Inventory Guidebook–2007. <http://www.eea.europa.eu/publications/EMEP/CORINAIR5/> (05. Nov. 2009)
- EPA. 2009. (U.S. Environmental Protection Agency), Life Cycle Assessment (LCA). <http://www.epa.gov/nrmrl/lcaccess/> (27. Oct. 2009)
- EPLC. 2009. European Platform on Life Cycle Assessment. <http://lca.jrc.ec.europa.eu/> (30. Oct. 2009)
- EuroFIR. 2009. (European Food Information Resource Network), Traditional Foods. <http://www.eurofir.net/public.asp?id=4292> (27. Oct. 2009)
- EuroGeoSurveys. 2007. Association of the Geological Surveys of Europe. <http://www.eurogeosurveys.org/> (20. May 2009)
- EUROHARP. 2004. <http://euroharp.org/index.htm> (20. May 2009)
- EUSOILS – European Soil Portal Home Page. 2009. <http://eusoils.jrc.ec.europa.eu/> (20. May 2009)
- Ferlay A., Chilliard Y., Martin B. 2007. Interim Report on the effect of vegetable oil and/or antioxidant supplementation of dairy diets on the nutritional composition, sensory quality and oxidative stability of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1a-1: 12 p.
- Foster C., Green K., Blenda M., Dewick P., Evans B., Flynn A., Mylan J. 2006. Environmental Impacts of Food Production and Consumption: A report to the Department for Environment, Food and Rural affairs. London. Manchester Buisness School & DEFRA
- Fox D.G., Tedeschi L.O., Tylutki T.P., Russel J.B., Van Ambrugh M.E., Chase L.E., Pell A.N., Overton T.R. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology*, 112: 29–78
- Fulladosa E., Serra X., Gou P., Arnau J. 2007. Interim report on the effect of potassium lactate and salt contents on the texture and flavour properties. IRTA. A report to the TRUEFOOD project N° D4.2.3-3: 8 p.
- Fulladosa E., Serra X., Gou P., Arnau J. 2009. Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, 82: 213–218
- Gaines W.L. 1927. The Energy Basis of Measuring Milk Yields. *Journal Of Animal Science*: 33–36
- Garcia N. 2009. "Details on deboning-salting-binding" IRTA (personal communication, 16. Feb. 2009)
- GEMIS 4.5 –Global Emission Model for Integrated Systems. 2009. ÖKO Institut e.V. <http://www.oeko-institut.de/service/gemis/en/index.htm> (28. Oct. 2009)
- González C.J., Overcash M. 2000. Energy sub-modules applied in life-cycle inventory of processes. *Clean Products and Processes*, 2: 57–66
- Gou P., Costa A., Arnau J. 2007. Interim Report on Conjoint Diffusivities of K-lactate and salt at different pH and temperatures. IRTA. A report to the TRUEFOOD project N° D4.2.3-1: 14 p.
- IPCC. 2001. Climate change 2001. The Scientific Basis. Contribution of the working group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press
- IPCC. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html> (12. Nov. 2009)
- IPCC. 2007. Intergovernmental Panel on Climate Change. Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Cambridge University Press
- ISO 14040. 2006. Environmental management -- Life cycle assessment -- Principles and framework

- LCA food database. 2007.
<http://www.lcafood.dk/> (20. May 2009)
- Lehuger S., Gabrielle B., Gagnaire N. 2009. Environmental impact of the substitution of imported soymeal with locally-produced rapeseed meal in dairy cow feed. *Journal of Cleaner Production*, 17: 616–624
- Lindfors L.G., Christiansen K., Hoffmann L., Virtanen Y., Juntila V., Hanssen O.J., Rønning A., Ekvall T., Finnveden G. 1995. Nordic Guidelines on Life-Cycle Assessment. Nord 20. Copenhagen, Nordic Council of Ministers
- Martin B., Graulet B., Pomiès D. 2007. Interim Report on the effect of once daily milking with and without calves on the nutritional composition of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1c-1: 9 p.
- Martin B., Ferlay A., Cornu A., Chilliard Y. 2008. Final Report on the effect of vegetable oil and/or antioxidant supplementation of dairy diets on the nutritional composition, sensory quality and oxidative stability of milk. IRTA. A report to the TRUEFOOD project N° D4.1.1a-3: 19 p.
- Martin B. 2009. "Meal composition in the oil supplementation experiment" INRA (personal communication, 03. Mar. 2009)
- Mattsson B., Cederberg C., Blix L. 2000. Agricultural land use in life cycle assessment (LCA): case studies of three vegetable oil crops. *Journal of Cleaner Production*, 8–4: 283–292
- MITERRA-EUROPE. 2009. SC ammonia. ALTERRA research institute.
<http://www.scammonia.wur.nl/UK/> (20. May 2009)
- Muñoz P. 2007. "Basic characteristics of cauliflower production in Spain" IRTA (personal communication, 09. Mar. 2007)
- Muñoz P. 2009. "Details on fertilizer treatment in the *Brassica* field experiment" IRTA (personal communication, 16. Mar. 2009)
- NRC – National Research Council. 2001. Nutrient Requirements of Dairy Cattle, seventh revised ed. National Academy Press, Washington, DC, USA
- OECD. 2001. Environmental Indicators for Agriculture, Methods and Results, Executive Summary.
<http://www.oecd.org/dataoecd/0/9/1916629.pdf> (30. Avg. 2007)
- Osojnik Črnič I.G., Marinšek Logar R. 2009. Identification of Environmental Impact Hot Spots in Traditional Food Production Lines. *Acta Agriculturae Slovenica*, 94, 1: in press
- Pieniaka Z., Verbeke W., Vanhonacker F., Guerrero L., Hersleth M. 2009. Association between traditional food consumption and motives for food choice in six European countries, research report. *Appetite*, 53, 1: 101–108
- Pomiès D. 2009. "Meal composition in the milking frequency experiment" (personal communication, 3. Mar. & 8. Apr. 2009)
- PURAC: lactic acid, lactates and gluconates. 2009.
http://www.purac.com/purac_com/ (20. May 2009)
- Roy P., Nei D., Orikasa T., Xu Q., Okadome H., Nakamura N., Shiina T. 2009. A review of life cycle assessment (LCA) on some food products. *Journal of Food Engineering*, 90: 1–10
- Schmidt A.C., Jensen A.A., Clausen A.U., Kampstrup O., Postlethwaite D. 2004. A Comparative Life Cycle Assessment of Building Insulation Products made of Stone Wool, Paper Wool and Flax. *International Journal of LCA*, 9,1: 53–66
- Serra X., Fulladosa E., Gou P., Arnau J. 2007. Interim report on the effect of novel deboning-salting-binding methodologies. IRTA. A report to the TRUEFOOD project N° D4.2.3-4: 14 p.
- Sibbel A. 2007. The sustainability of functional foods. *Social Science & Medicine*, 64, 3: 554–561
- Sjaunja L.O., Baevre L., Junkkarinen L., Pedersen J., Setälä J. 1991. A Nordic Proposal for an Energy Corrected Milk (ECM) Formula. EAAP Publication No. 50: 156–157
- Smith K.A., Jackson D.R., Pepper T.J. 2001a. Nutrient losses by surface run-off following the application of organic manures to arable land. 1. Nitrogen. *Environmental Pollution*, 112, 1: 41–51
- Smith K.A., Jackson D.R., Withers P.J.A. 2001b. Nutrient losses by surface run-off following the application of organic manures to arable land. 2. Phosphorus. *Environmental Pollution*, 112, 1: 53–60
- Tomassen M.A., De Boer I.J.M. 2005. Evaluation of indicators to assess the environmental impact of dairy production systems. *Agriculture, Ecosystems and Environment*, 111: 185–199
- Tomassen M.A., Dolman M.A., van Calster K.J., de Boer I.J.M. 2009. Relating life cycle assessment indicators to gross value added for Dutch dairy farms. *Ecological Economics* 68, 8–9: 2278–2284
- TRUEFOOD. 2009. (Traditional United Europe Food), Project Results.
<http://www.truefood.eu/page.asp?ID=23> (12. Nov. 2009)
- Vink E.T.H., Rábago K.R., Glassner D.A., Gruber P.R. 2003. Applications of life cycle assessment to NatureWorks™ polylactide (PLA) production. *Polymer Degradation and Stability*, 80: 403–419
- Vink E.T.H., Glassner D.A., Kolstad J.J., Wooley R.J., O'Connor R.P. 2007. The eco-profiles for current and near-future NatureWorks' polylactide (PLA) production. *Industrial Biotechnology*: 58–81
- Wenzel H., Hauschild M., Alting L. 1998. Environmental assessment of products. Volume2: Scientific background. Springer: 584 p.
- Williams A.G., Audsley E., Sandars D.L. 2006. Determining the environmental burdens and resource use in the production of agricultural and horticultural commodities. Main report, DEFRA research project IS0205. Bedford. Cranfield University & DEFRA
- Zah R., Böni H., Gauch M., Hirschler R., Lehmann M., Wäger P. 2007. Ökobilanz von Energieprodukten: Ökologische Bewertung von Biotreibstoffen. Bern, EMPA: 206 p.

PREBAVNA MIKROBIOTA KOT DEJAVNIK PRI RAZVOJU DEBELOSTI

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Prebavna mikrobiota kot dejavnik pri razvoju debelosti

Porast debelosti v moderni družbi je povezan z večjo pojavnostjo z debelostjo povezanih bolezni in predstavlja veliko finančno breme za javno zdravstvo. Pomembno odkritje na področju mikrobiologije prebavnega trakta sesalcev je povezano z vlogo prebavne mikrobiote pri razvoju debelosti. Z novimi molekularnimi metodami in poskusi z gnotobiotskimi živalmi so do neke mere pojasnili udeležbo prebavne mikrobiote pri uravnavanju telesne mase in energijskega ravnovesja gostitelja. Prebavna mikrobiota vpliva na vnos hranil in porabo energije iz hrane in pospešuje shranjevanje le-te v maščobna tkiva s procesi fermentacije, olajšane absorpcije in tudi z vplivom na izražanje gostiteljevih genov (protein Fiaf) ter na aktivnost gostiteljevih encimov (proteinska kinaza AMPK). Pri debelih miših in ljudeh je prebavna mikrobiota dokazano bolj učinkovita pri izkoriščanju energije iz hrane kot pri suhih, osebkih. Obstajajo značilne razlike v sestavi mikrobne združbe glede na debelost oz. suh fenotip. V prebavilih debelih živali in ljudi se dosledno kaže povišan delež predstavnikov bakterij iz debla *Firmicutes* na račun zmanjšanja predstavnikov debla *Bacteroidetes*, obe prevladujoči debeli pa v prebavilih sesalcev skupaj predstavljata do 90 % vseh bakterij. Izkazalo se je, da je prebavna mikrobiota udeležena tudi pri patofiziologiji debelosti preko dejavnikov, kot je mikrobni LPS. Rezultati raziskav kažejo, da lahko spremembe deleža maščob v hrani vplivajo na sestavo mikrobne združbe ter da te spremembe vplivajo na pojavnost metabolnih bolezni. Odpira se novo področje manipulacije prebavne mikrobiote za zdravljenje debelosti in z njo povezanih bolezni.

Ključne besede: mikrobiologija / prebavni trakt / debelo črevo / mikrobiota / *Bacteroidetes* / *Firmicutes* / debelost / ITM / genomika / metagenomika

Gut microbiota as a factor in obesity development

The increased prevalence of obesity in modern society is associated with incidence of obesity related diseases and represents a financial burden on public health. Important discovery in the field of microbial ecology of the gut was the possible involvement of the gut microbiota in obesity development. Using new molecular techniques and gnotobiotic animal models has revealed the relation between the regulation of body mass and energy balance of the host with the microbial community of the gut. Gut microbiota affects nutrient intake, facilitate the extraction of energy from food and promote storage of the calories in host adipose tissue through processes of fermentation, absorption and through the effect on the expression of host genes (e.g. Fiaf) and the activity of host enzymes (e.g. AMPK). In obese mice and humans the gut microbiota is clearly able to obtain energy from food more effectively as in the lean subjects. There are significant differences in the composition of microbial communities in relation to fat vs. lean phenotype. In the gut of obese animals and humans the increased proportion of the *Firmicutes* at the expense on *Bacteroidetes* was consistently detected. Both are the dominant bacterial groups in mammalian gastrointestinal tract, accounting together for 90% of all bacteria. It has been shown that gut microbiota is involved also in pathophysiology of obesity through factors such as microbial LPS. Existing results show that high fat diet can affect the composition of microbial community in the gut and that these changes can further affect the incidence of metabolic disease. This evidence potentially opens a new field of manipulation of the gut microbiota as a new strategy to treat obesity and related diseases.

Key words: microbiology / alimentary tract / hindgut / microbiota / *Bacteroidetes* / *Firmicutes* / obesity / BMI / genomics / metagenomics

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1 EPIDEMIJA PREKOMERNE DEBELOSTI

Debelost je stanje telesa, pri katerem je delež maščob prekomerno povečan in zato predstavlja breme za telo. Povečevanje števila debelih ljudi je značilen pojav v družbah razvitega dela sveta, kjer je hrane v obilju in hkrati prevladuje življenjski slog z nezadostno telesno aktivnostjo. Prekomerno kopičenje maščobnih tkiv ima vpliv na izgled in zdravje telesa ter lahko vodi v razvoj bolezni, zato je prekomerna debelost opredeljena kot bolezensko stanje. Debelost je ključna pri nastanku in napredovanju nekaterih kroničnih nenalezljivih bolezni, kot so bolezni srca in ožilja, sladkorna bolezen in nastanek malignih novotvorb (Calle in Thun, 2004). Dokazana je bila jasna povezava med prekomerno telesno maso in povišanimi vrednostmi holesterola in sladkorjev v krvi, kar posledično povečuje pojavnost srčno-žilnih bolezni. Od sedmih pomembnih dejavnikov tveganja za nastanek kroničnih nenalezljivih bolezni jih je vsaj pet tesno povezanih s telesno nedejavnostjo in nezdravo prehrano. V gospodarsko razvitih državah zahodnega sveta, predvsem v ZDA, kjer je opravljenih tudi največ študij, povezanih z debelostjo, je pretirana debelost na sedmem mestu najpogostejših dejavnikov tveganja za razvoj bolezni, v razvitih državah pa je med vzroki za smrt debelost, ki je posledica pretiranega hranjenja na drugem mestu, takoj za zlorabo tobaka. V sistemu zdravstvenega zavarovanja ZDA spremljajo in vrednotijo strošek, ki ga predstavljajo posamezne bolezni, in v letu 2008 naj bi ZDA za bolezni, ki so posledica debelosti, porabile kar 45 milijard dolarjev.

Telesno maso ocenjujemo z indeksom telesne mase (ITM). Mejne vrednosti ITM za prekomerno debelost so v različnih državah med 25 in 30 kg/m². Vloga maščobnih tkiv v zdravem telesu je shranjevanje energije, del teh tkiv pa sodi med endokrine žleze, ki uravnavajo imunski sistem in sodelujejo pri sami regulaciji shranjevanja energije. Telo sesalcev je evolucijsko bolj zaščiteno pred stradanjem kot pred pretiranim obiljem, saj je izguba telesne mase za organizem nevarnejša od pretirane debelosti. Povečanje telesne mase je rezultat interakcij med gensko zasnovo ter socialnimi, kulturnimi in okoljskimi dejavniki in je večinoma posledica obilja.

Ocene vpliva genotipa na debelost so med 6 in 85 % (Yang in sod., 2007), s samimi genetskimi razlikami med ljudmi pa ne moremo pojasniti porasta števila pretirano debelih ljudi v tako kratkem obdobju, kot smo temu priča v zadnjih desetletjih. Genetsko pogojena debelost je večinoma poligenske oblike, pri kateri je udeleženo večje število genov (Rankinen in sod., 2002), katerih učinki so manjši. Debelost, ki je posledica mutacije enega gena, je v populaciji redka, so pa posledice te mutacije večje (O'Rahilly, 2002). Najbolj raziskana je mutacija v genu za peptidni hormon leptin. Leptin večinoma nastaja v

maščobnih celicah, adipocitah, in igra pomembno vlogo v metabolizmu glukoze in maščobnih kislin. Količine leptina so proporcionalne količini telesnih maščob in do povečanega izražanja leptina pride ob povečanem številu adipocit (Couturier in sod., 2007). Mutacije v genu za leptin (*ob*) ali v genu za leptinski receptor (*lepr*) v populaciji so redke, zanje so uveljavljena in uspešna zdravljenja z rekombinantnim leptinom, hkrati pa so tudi osnova mišjega modela, na katerem potekajo raziskave tako debelosti kot z njo povezanih bolezni. Zaradi majhne pojavnosti te mutacije v populaciji pa so pomembne študije na drugih modelih, ki ne izhajajo iz monogensko pogojene genetske okvare.

Kot smo že omenili, z genetiko ne moremo pojasniti hitrega povečevanja števila prekomerno debelih ljudi po celem svetu. Svetovna zdravstvena organizacija je prepoznala prekomerno debelost kot epidemijo leta 1997 in osem let kasneje so podatki kazali, da je najmanj 400 milijonov ljudi po vsem svetu predebelih.

1.1 STANJE DEBELOSTI V SLOVENIJI

Tudi v Sloveniji se delež čezmerno debelih oseb povečuje in s tem zdravstveni problemi, povezani s preobilno prehrano in nezadostno telesno dejavnostjo. V letu 2002 je bilo do 40 % vseh smrti v Sloveniji posledica bolezni srca in ožilja. Po ocenah pristojnih inštitucij ekonomsko breme bolezni, ki so povezane s prekomernim hranjenjem, že presega zmogljivosti zdravstvenih zavarovanj. Raziskave odrasle populacije so sicer redke, kažejo pa na naraščanje deleža čezmerno hranjenih (ITM med 25 in 30) ter deleža prekomerno debelih (ITM nad 30) ljudi. Glede na izsledke raziskav iz let 1996/97, 2001 ter 2002/2003 so bili deleži čezmerno prehranjenih Slovencev med 55 in 62 %, deleži prekomerno debelih pa med 15 in 20 % (Ministrstvo za zdravje RS, 2007). Razširjenost debelosti v Sloveniji je primerljiva z drugimi evropskimi državami, porast debelosti pa se kaže tudi pri otrocih in pubertetnikih (Avbelj in sod., 2005). V okviru Mednarodne zdravstvene organizacije je Evropska unija v letu 2006 podpisala deklaracijo za preprečevanje naraščanja debelosti s ciljem zaustaviti epidemijo debelosti do leta 2015. Ministrstvo za zdravje RS je dokument podprlo in se s tem zavezalo k reševanju te problematike.

Pri pridobivanju energije iz hrane imajo bolj ali manj pomembno vlogo tudi mikroorganizmi, ki naseljujejo prebavni trakt, t.i. prebavna mikrobiota. Rezultati novejših študij vpliva mikrobne združbe v prebavilih so dali slutiti, da ima prebavna mikrobiota pomembno vlogo tudi pri nastanku debelosti. S tem namenom se je pomemben del raziskav, ki so osredotočene v debelost, usmeril v preučevanje vloge mikrobne združbe v preba-

vilih. V pričujočem prispevku se bomo omejili na vlogo prebavne mikrobiote pri razvoju debelosti v primeru monogastričnih sesalcev, med katere spada tudi človek.

2 MIKROBNA ZDRUŽBA V PREBAVNEM TRAKTU SESALCEV

Mikrobiologija prebavnega trakta je področje, ki se je v zadnjem desetletju zaradi uveljavitve ter znižanja stroškov molekularnih metod izjemno razmahnilo. Pred prihodom t.i. druge generacije molekularnih tehnik je bilo proučevanje mikrobov v prebavilih sprva omejeno na tiste, ki jih je bilo mogoče gojiti, kasneje pa na omejeno število tistih, ki jih je bilo mogoče zaznati z molekularnimi tehnikami prve generacije. Večina molekularnih študij temelji na obsežnem odkrivanju in/ali nabiranju nukleotidnih zaporedij genov za 16S rRNA, za katere velja, da so primerni molekularni označevalci (ang. *marker*), ker so prisotni v vseh organizmih in so zadostno ohranjeni za poravnave ter dovolj različni za filogenetske analize. Po letu 2003 se je z razvojem metod hitrega in masovnega sekvenciranja (Leamon in sod. 2003; Margules in sod., 2005) nabiranje novih sekvenc iz prebavne mikrobiote še pospešilo ter omogočilo razmah študij primerjalne genomike.

V prebavnem traktu sesalcev je ocenjeno število mikrobnih celic med 10^{13} in 10^{14} ($\sim 10^{11-12}$ celic/ml prebavne vsebine). Večina prebavnih mikroorganizmov pri monogastričnih živalih, kamor sodi tudi človek, naseljuje zadnji del prebavnega trakta, to je debelo črevo ali kolon, ki ga uvrščamo med ekosisteme z največjim številom mikrobnih celic na prostorninsko enoto (Bäckhed in sod., 2005). Med prisotnimi mikroorganizmi prevladujejo predvsem po Gramu pozitivne bakterije, arheje in evkarionti pa so v manjšini: v prebavilih miši so tako deleži bakterij med 90–96 %, do 2 % mikrobne združbe zastopajo arheje, evkarionti predstavljajo od 1–7 % ter virusi 1 % mikrobne združbe (Turnbaugh in sod., 2006). V prebavnem traktu je pestrost mikrobne združbe relativno majhna v primerjavi z ekosistemi, kjer prav tako prihaja do razgradnje organske snovi, kot so na primer tla (Ley in sod., 2006b). V prebavilih ljudi in miši kar 90 % vseh bakterijskih filotipov prebavne mikrobiote pripada debloma *Firmicutes* in *Bacteroidetes*, to je le dvema od sedemdesetih bakterijskih debel, in sicer v razmerju 60–80 %: 20–40 % (Ley in sod., 2005, 2006a; Eckburg in sod., 2005). Pestrost mikrobne združbe se kaže predvsem na nižjih taksonomskih nivojih, to je vrstah in podvrstah, kar naj bi bila posledica gostiteljske selekcije in koevolucije v relativno stabilni ekološki niši (Dethlefsen in sod., 2007). Ocenjeno število bakterijskih »molekularnih« vrst

v debelem črevesu odraslega človeka je okrog 1000, od teh naj bi jih bilo manj kot 70 ubikvitarnih (Dore, 2009).

Do začetne okužbe sesalskega prebavila pride ob porodu, na sestavo prebavne mikrobiote, ki se bo v prebavilu nato vzpostavila, pa imajo vpliv tako genotip gostitelja kot okoljski dejavniki (Camp in sod., 2009), vključno s fiziološko anatomskimi spremembami prebavnega trakta, do katerih pride med razvojem (Berg 1996; Palmer in sod., 2007). Raziskave vpliva različnih dejavnikov na sestavo prebavne mikrobiote, ki so bile opravljene v preteklosti, temeljijo na različnih metodah in rezultati teh študij so si zato pogosto nasprotujoči. Zaradi načina okužbe predvidevamo, da si bosta združbi prebavne mikrobiote matere in njenih otrok precej podobni. To so zaznali Turnbaugh in sod. (2009) pri pregledu prebavne mikrobiote enojajčnih in dvojajčnih dvojčkov ter njihovih mater. Palmer in sod. (2007) v nasprotju s tem pri pregledu prebavne mikrobiote v fecesu otrok in njihovih mater niso zaznali večjih razlik od prebavne mikrobiote drugih odraslih ljudi. Ley in sod. (2005) so na modelu suhih in debelih linijah miši ugotovili, da je sestava prebavne mikrobiote med materami in mladiči enaka, razlikuje pa se zastopanost posameznih skupin in le-ta je bila odvisna od genotipa (debel-suh). Vpliv gostitelja na sestavo prebavne mikrobiote so zaznali tudi pri prenosu prebavne mikrobiote iz prebavil rib cebric (*Danio rerio*) v prebavila gnotobiotskih sterilnih miši. Po prenosu se je sestava mikrobne združbe v prebavilu okuženih miši v času spremenila in postala bolj podobna prebavni mikrobioti običajnih miši (Rawls in sod., 2006). Sicer naj bi sestava prebavne mikrobiote pri istih osebkih v času ostajala enaka (Zoetendal in sod., 2001) in naj bi glede na okoljske dejavnike prihajalo do sprememb predvsem v deležu prisotnih skupin (Ley in sod., 2005). O prožnosti ali stabilnosti prebavne mikrobiote govori študija Dethlefsena in sod. (2008), v kateri so ugotovili, da se je po štirih tednih po prenehanju jemanja antibiotika v prebavilih ljudi vzpostavila enaka združba prebavne mikrobiote kot pred jemanjem, ne glede na to, da so antibiotiki sprva znižali mikrobno pestrost.

Kljub temu nekatere opravljene študije kažejo, da se sestava prebavne mikrobiote spreminja v odvisnosti od tipa hrane (Ley in sod., 2006a; Nadal in sod., 2009) in se do neke mere spreminja tudi s starostjo (Mueller in sod., 2006; Mariat in sod., 2009).

3 RAZLIKE V SESTAVI PREBAVNE MIKROBIOTE DEBELIH IN SUHIH OSEBKOV

Novejše študije so jasno pokazale, da obstaja korelacija med povišanim deležem telesnih maščob in strukturo mikrobne združbe v prebavilih miši (Ley in sod.,

2005), podgan (Mozes in sod., 2008), prašičev (Guo in sod., 2009) in ljudi (Ley in sod., 2006a; Collado in sod., 2008; Nadal in sod., 2009). Pri vseh so zaznali statistično značilno spremembo v relativni zastopanosti predstavnikov bakterijskih debel *Bacteroidetes* in *Firmicutes* kot povišanje deleža firmikutov pri debelih osebkih.

Spremembe v razmerju predstavnikov firmikutov in bakteroidet so prvič zaznali v prebavnem traktu debelih (linija *ob/ob* z okvaro v genu za leptin) in suhih (linija *+/+*) miši (Ley in sod., 2005), in sicer je bilo pri debelih miših do 50 % več firmikutov. V nadaljevanju so sledili spremembi deleža firmikutov in bakteroidet v fecesu prekomerno debelih ljudi med enoletnim programom hujšanja ob spremembi iz visoko kalorične v nizko kalorično dieto (Ley in sod., 2006a). Prebavna mikrobiota se je spreminjala skupaj z zmanjšanjem telesne mase. Skupni delež obeh bakterijskih skupin je ostal enak, spremenilo pa se je razmerje med prevladujočima skupinama v prid bakteroidet, in sicer iz 3 % na začetku na 15 % celotne bakterijske združbe ob koncu poskusa. Podobno se je med hujšanjem devetintridesetih prekomerno debelih pubertetnikov spremenila zastopanost izbranih bakterijskih skupin (Nadal in sod., 2009). Ob znižanju ITM so v fecesu zaznali zmanjšanje števila nekaterih skupin iz debla *Firmicutes* ter zvišanje deleža bakterij iz debla *Bacteroidetes*.

Krmljenje z visoko kalorično dieto je tudi pri podganah spremljala sprememba v bakterijski združbi prebavne mikrobiote, kjer so opazili značilno zmanjšanje števila predstavnikov iz debla *Bacteroidetes* in povečanje števila rodov enterokokov in laktobacilov iz debla *Firmicutes* pri debelih podganah v primerjavi s suhimi (Mozes in sod., 2008).

Nedavno so Turnbaugh in sod. (2009) v metagenomski študiji pregledali ogromno število celotnih in delnih sekvenc bakterijskih genov za 16S rRNA v fecesu debelih in suhih ljudi ($n=154$). Ugotovili so, da je prebavni mikrobiom družinskih članov podoben in da je proučevanim osebkom bolj kot nabor bakterijskih vrst skupen nabor genov. Zaznali so tudi značilno manjšo pestrost na ravni bakterijskih rodov v fecesu debelih ljudi v primerjavi s suhimi in zato predpostavili, da pretirana debelost bakterijsko raznolikost zmanjšuje.

Razlike v razmerju predstavnikov debel *Firmicutes* in *Bacteroidetes* so odkrili tudi pri različnih starostnih skupinah ljudi. Mariat in sod. (2009) so pregledali zastopanost bakterijskih skupin *Clostridium leptum*, *Clostridium coccoides*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus* in *Escherichia coli* v fecesu otrok (3–10 mesecev), odraslih (25–45 let) ter starejših ljudi (70–90 let) z metodo kvantitativnega PCR (qPCR). Za prebavno mikrobioto majhnih otrok je bilo značilno prevladovanje bifidobakterij in tudi bakterije iz vrste *E. coli* so bile pri majhnih

otročih na nivoju dominantne skupine. Pri odraslih so prevladovali firmikuti in bakteroidete, pri starejših ljudeh pa se je ponovno statistično značilno povečal delež *E. coli*. Razmerje med številom celic firmikutov in bakteroidet se je med starostnimi skupinami razlikovalo in je bilo pri otrocih 0,4, pri odraslih 10,9 ter 0,6 v skupini starejših ljudi. Kalliomaki in sod. (2008) so spremljali število glavnih bakterijskih skupin prebavne mikrobiote pri odraščajočih otrocih (od rojstva do 7. leta starosti) in primerjali otroke, ki so kasneje postali prekomerno debeli in otroke z normalno telesno maso. Predlagali so, da je mogoče po zastopanosti bakterijskih skupin v prebavni mikrobioti sklepati na kasnejšo nagnjenost k debelosti. Glavne razlike so odkrili v številu bifidobakterij in streptokokov, prvih je bilo pri otrocih, ki so kasneje postali debeli, manj, streptokokov pa več.

4 VLOGA PREBAVNE MIKROBIOTE PRI PRIDOBIVANJU ENERGIJE IZ HRANE

Kakšno vlogo ima povišanje relativnega deleža predstavnikov debla *Firmicutes* v prebavilu debelih ljudi in živali, kakšno vlogo številčnejši predstavniki debla *Bacteroidetes* pri suhih, ter na kakšen način poteka selekcija, še ni jasno. Predstavniki debla *Bacteroidetes* v prebavilih fermentirajo ogljikove hidrate, porabljajo dušikove spojine ter so udeleženi v biotransformaciji žolčnih kislin. S pomočjo bakteroidet in njihovih encimov se v prebavilih ponovno dekonjugira tudi do 95 % konjugiranih žolčnih kislin, ki se lahko nato kot take vrnejo v telo. Večina bakteroidet je »zmerno«
saharolitičnih, hidrolizirajo tudi kompleksne rastlinske polisaharide, kot so ksilan, pektin in celuloza, in imajo za to veliko število genov za različne glikozilaze. Pri nekaterih vrstah iz rodu *Bacteroides* so ugotovili, da so hidrolazni encimi nameščeni na notranji membrani, njihovo izražanje pa je regulirano glede na prisotne polisaharide, zaradi česar naj bi se bakteroidete lažje prilagajale prisotnim virom substrata. V deblu *Firmicutes* spadajo klostridiji, bacili, laktobacili in molikuti. V zadnjem delu prebavnega trakta človeka in živali so prisotni predvsem firmikuti iz razreda klostridijev in laktobacilov, prvih pa je bistveno več in jim pripisujemo pomembnejšo vlogo. Pri fermentaciji polisaharidov uporabljajo podoben sistem membranskih proteinov kot bakteroidete, le da jim substrata ni potrebno prenesti v periplazmo.

Da bi lahko razumeli funkcijo tako ene kot druge skupine v prebavnem traktu ter kakšen vpliv bi predstavniki bakteroidet in firmikutov lahko imeli pri razvoju debelosti, so opravili študije okužbe sterilnih gnotobiotičnih miši z izbranimi sevi. Mahowald in sod. (2009) so gnotobiotske sterilne miši kolonizirali z dvema sevoma,

s predstavnikom firmikutov iz vrste *Eubacterium rectale* in s predstavnikom bakteroidet iz vrste *Bacteroides thetaiotaomicron*. Slednji sodi med dobro proučevane bakteroidete (Bäckhed in sod., 2004, 2005). Zanj je značilno ogromno število zapisov za različne glikozidne hidrolaze (226) in polisaharidne liaze (15) (Bjursel in sod., 2006). Vsi ti proteini tvorijo t.i. glikobiom, celoten set genov oz. njihovih encimov, ki so udeleženi pri izkoriščanju ogljikovih hidratov. S transmembranskimi proteini zaznavajo različne polisaharide in jih prenašajo v periplazmo, različne hidrolaze pa omogočajo njihovo razgradnjo do monosaharidov. Za *E. rectale* je značilno manjše število encimov, ki razgrajujejo polisaharide v primerjavi z obsežnim glikobiomom *B. thetaiotaomicron*. *E. rectale* je poznan tudi kot producent maslene kisline iz očetne kisline in njegova prisotnost povečuje ekspresijo transporterjev maslene kisline pri miših. Pokazali so, da se predstavniki *B. thetaiotaomicron* hranijo na stenah prebavnega trakta in imajo zvišano raven izražanja predvsem za encime, ki cepijo mukozne snovi in ki so nedostopne *E. rectale*, do očetne kisline. Prisotnost predstavnikov obeh združb omogoča boljši izplen prisotnih substratov.

Čeprav v prebavnem traktu dominirajo predstavniki bakterij, imajo tudi arheje pomembno vlogo pri povečanju izplena energije iz fermentacije hrane. Večinoma so v prebavilih vsejedi sesalcev prisotne hidrogenotrofne metanogene arheje, ki z odstranjevanjem vodika omogočajo ostalim mikroorganizmom proizvajati več očetne in maslene kisline, ki se nato absorbirata skozi epitelne celice črevesa. Metanogene arheje z odstranjevanjem vodika preprečujejo njegovo kopičenje kot tudi kopičenje končnih produktov fermentacije, kot je mravljična kislina. Šele nastali metan predstavlja končni odliv tako energije kot elektronov. Najpogostejše zastopana arheja pri človeku je vrsta *Methanobrevibacter smithii*, ki lahko predstavlja po nekaterih študijah celo več kot 10 % celotne mikrobne populacije v prebavilu (Samuel in sod., 2007).

Za boljše razumevanje doprinosa arhej k celokupnemu izplenu iz fermentacije v prebavnem traktu sta Samuel in Gordon (2006) sterilne gnotobiotske miši okužila s sevoma iz vrst *Methanobrevibacter smithii*, *Bacteroides thetaiotaomicron* ter mešanico obeh. Največji izkoristek iz hrane ter povišano lipogenezo sta odkrila ob prisotnosti obeh in zaznala do 1000-kratno povečanje števila predstavnikov obeh vrst, če sta rasli skupaj. Ugotovila sta, da *Methanobrevibacter smithii* vpliva na ekspresijo genov v *B. thetaiotaomicron*, in sicer tako, da ta svoj metabolizem preusmeri v razgradnjo fruktoznih polisaharidov, ki jih cepijo do mravljične kisline, vira energije za metanogenega partnerja. Mikrobi v prebavnem traktu torej ne vplivajo le na gostitelja, temveč tudi eden na drugega, v kolikor so med njimi skupni intermediati. Pri koloniza-

ciji prebavnega trakta miši s sevoma iz vrst *Desulfovibrio piger* in *B. thetaiotaomicron* do povečanja lipogeneze v miši ali do povečanja števila obeh skupin mikroorganizmov ni prišlo (Samuel in Gordon, 2006).

5 PREBAVNA MIKROBIOTA KOT METABOLNI ORGAN GOSTITELJA

Prebavna mikrobiota je torej prepoznana kot pomemben dejavnik pri razgradnji hrane, povečevanju dostopnosti hranil, vira energije ter pri sintezi vitaminov. Funkcije prebavne mikrobiote so bile razjasnjene s poskusi na gnotobiotskih laboratorijskih živalih, predvsem miših (Berg, 1996). Prebavna mikrobiota se je izkazala za nujno potrebno pri vzdrževanju normalne funkcije črevesa, kot je razgradnja in sprejem hranil, ter pri razvoju imunosti. Med njene dodatne funkcije danes štejemo še regulacijo metabolizma in biološke dostopnosti zdravil, kot so ksenobiotiki, razgradnjo dietetičnih toksinov, obnovo epitelnih celic, postavitve in aktivacije imunskega sistema, vpliv na velikost srca ter celo na gibalne spretnosti (Turnbaugh in sod., 2007).

Zaradi naštetih vplivov prebavne mikrobiote na samega gostitelja prebavno mikrobioto pogosto omenjamo kot metabolni organ, ki opravlja funkcije, ki jih sesalci med evolucijo nismo razvili, celotni set mikrobnih genov v prebavilu pa kot prebavni mikrobiom. V sklopu celotnega prebavnega mikrobioma naj bi obstajal, kot že omenjeno, poseben nabor genov, tako imenovano jedro mikrobioma, ki so nujno potrebni za zdravje gostitelja (Turnbaugh in sod., 2009). Rezultate za potrditev te hipoteze so raziskovalci pridobili predvsem z metodami masovnega sekvenciranja, s pomočjo katerih je mogoče zbrati veliko število sekvenc, jih nato obdelati z orodji primerjalne metagenomike ter na podlagi rezultatov primerjav sklepati, katere metabolne poti so v določenem primeru bolj izražene (Tringe in sod., 2005). Gill in sod. (2006) so pregledali veliko število mikrobnih genov v fecesu dveh oseb in primerjali zapise bakterijskih genov za encime z geni gostitelja. Na podlagi rezultatov primerjav so nato sklepali, katere metabolne funkcije mikroorganizmi opravljajo v prid gostitelju. Zasledili so predvsem povišano število genov za metabolizem glikanov, aminokislin in ksenobiotikov.

6 VLOGA PREBAVNE MIKROBIOTE PRI RAZVOJU DEBELOSTI

6.1 VLOGA PREBAVNE MIKROBIOTE PRI URAVANJANJU SKLADIŠČENJA MAŠČOB

Da imajo mikroorganizmi pomembno vlogo pri razvoju debelosti, so med prvimi pokazali Bäckhed in sod. (2004, 2005). V nizu poskusov so ugotovili, da prebavni mikrobi na več načinov povečujejo energetski izkoristek iz hrane. V začetnih poskusih so primerjali telesno maso in delež maščob v telesu običajnih in sterilnih gnotobiot- skih miši, ki so zaužile enako količino hrane. Pri običaj- nih miših so izmerili do 40 % več maščob v telesu kot pri njihovih vrstnicah, ki so rasle brez mikrobov v prebav- nem traktu. V naslednjem koraku so v sterilne gnotobi- otske miši prenesli prebavno mikrobioto običajnih miši. Po okužbi so gnotobiotske miši pridobile do 60 % več tele- sne maščobe brez povečanja vnosa hrane ali zmanjšanja telesne aktivnosti. Poskus so nato ponovili z gensko spre- menjenimi mišmi z okvaro v genu za leptin (linija *ob/ob*). S prebavno mikrobioto debelih *ob/ob* miši so okužili običajne sterilne miši ter ponovno zaznali značilno večje povečanje telesne mase v primerjavi z mišmi, ki so bile okužene s prebavno mikrobioto običajnih mišk. Postavili so hipotezo, da mikrobiota debelim mišim omogoča več- ji izkoristek energije iz sicer slabo razgradljivih polisaharidov (Bäckhed in sod., 2005).

Mehanizem v ozadju je kasneje osvetlila ista skupina raziskovalcev. Ugotovili so, da so sterilne miši zaščitene pred razvojem debelosti, do katere pride zaradi načina prehranjevanja, vsaj z dvema med sabo ločenima meha- nizmoma.

- Prebavna mikrobiota lajša absorpcijo monosaha- ridov, glukoze in maščobnih kislin, in povečuje lipogenezo v jetrih preko odziva dveh signalnih proteinov, ChREBP in SREBP-1c. Povišane ravni inzulina zaradi aktivacije transkripcijskih fak- torjev, kot sta ChREBP in SREBP-1c, zvišujejo nastanek 3-gliceridov v jetrih, le-ti se sproščajo v krvni obtok in se nato kopičijo v adipocitnih celicah maščobnih tkiv v procesu lipogeneze.
- Prebavna mikrobiota inhibira sintezo proteina Fiaf (fasting-induced adipocyte factor), ki je značilen za prebavila in v nizu reakcij povečuje oksidacijo shranjenih maščob.

Zadnje so sledili na gensko spremenjenih miškah z mutacijo v genu za Fiaf. Sam Fiaf deluje tako, da zavira aktivnost lipoproteinskih lipaz (LPL) in s tem povečuje sproščanje maščobnih kislin iz adipocit. Zaviranje izra- žanja faktorja Fiaf vodi v povišanje aktivnosti LPL ter posledično v shranjevanje maščobnih kislin v maščobna tkiva. Pokazali so tudi, da Fiaf poveča oksidacijo maščob-

nih kislin v mitohondriju s povečevanjem izražanje ge- nov, ki kodirajo regulatorje oksidacije maščobnih kislin (PGC-1 α).

Na vpliv prebavne mikrobiote pri skladiščenju ener- gije dodatno kažejo izmerjene aktivnosti encima AMPK, proteinske kinaze, ki sodeluje v uravnavanju energetske homeostaze v celicah. Do aktivacije AMPK pride ob ce- ličnem stresu. V kaskadi reakcij se AMPK fosforilira in kot taka deluje na encime in na izražanje genov, ki so ude- leženi v procesih, v katerih nastaja ATP, kot so glikoliza, oksidacija maščobnih kislin, vnos glukoze itd. Povišane koncentracije fosforiliranega encima so bile značilne za sterilne gnotobiotske miši, medtem ko je prisotnost pre- bavne mikrobiote količino fosforilirane AMPK zmanjša- la (Bäckhed in sod., 2007). Razlogi za povišane koncen- tracije fosforilirane kinaze v običajnih miškah niso jasni, rezultati omenjene študije pa so pokazali, da prebavna mikrobiota vpliva na porabo energije preko fermentacije in absorpcije, kot tudi preko vpliva na ekspresijo gostite- ljevih genov in aktivnost gostiteljevih encimov.

Turnbaugh in sod. (2006) so predlagano hipotezo Bäckheda in sod (2004, 2005) preverili s primerjalno me- tagenomiko. Pregledali so prisotnost genov v mikrobio- mu debelih (*ob/ob*) in suhih (+/+) miši in iskali povezavo med gostiteljevim fenotipom (debelostjo/suhostjo) in izraženimi metabolnimi potmi prebavne mikrobiote. V fecesu debelih miši so odkrili povišane deleže genov bak- terij iz debla *Firmicutes*, kar se sklada z rezultati raziskav strukture združbe prebavne mikrobiote v miših (Ley in sod., 2005) in drugih živalih. V prebavilih debelih miši so izmerili večje koncentracije končnih produktov fermen- tacije, kot sta maslena in očetna kislina, in manj kalorij v izločenem fecesu, primerjalna metagenomska analiza pa je dodatno pokazala povišane deleže genov, ki kodirajo encime za razgradnjo kompleksnih polisaharidov. Glede na delež genov so sklepali, da je mikrobiom debelih mišk bolj sposoben razgraditi kompleksnejše polisaharide do kratkoveržnih maščobnih kislin in tako pridobiti več energije.

Vsi ti rezultati kažejo, da prebavni mikrobiom s svojim setom genov predstavlja dejavnik, ki skupaj z ge- notipom ter načinom življenja gostitelja igra vlogo pri razvoju debelosti. Odprla so se nova vprašanja, na katera stroka vztrajno išče odgovor:-

6.2 VLOGA PREBAVNE MIKROBIOTE PRI RA- ZVOJU Z DEBELOSTJO POVEZANIH BOLE- ZNI

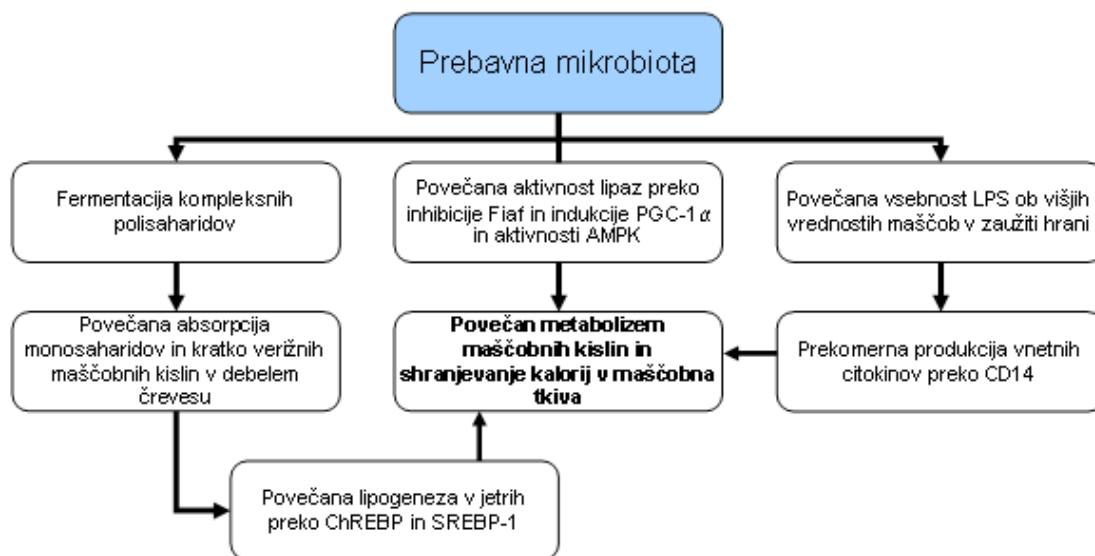
Za debelost so med drugim značilne povišane vre- dnosti vnetnih faktorjev v krvi, kar so pokazali na pod- ganah, miših in tudi pri ljudeh (Cani in sod., 2009). Do

prekomerne produkcije vnetnih citokinov prihaja predvsem v adipocitah in to vodi v kronično sistemsko vnetje. Hkrati je pri debelih osebkih prisotna večja vsebnost lipidov v krvi, kar skupaj z vnetjem dodatno zmanjšuje imunost, iz česar se razvije inzulinska rezistenca (Wellen in sod., 2005).

Cani in sod. (2007a, 2008) je zanimal vpliv prebavne mikrobiote na debelost in razvoj inzulinske rezistence. V poskusih z mišmi, ki so jih hranili s krmo z visokim deležem maščob, so pokazali, da takšna krma povečuje t.i. endotoksimijo (količino LPS v krvi) in vpliva na sestavo mikrobne združbe. Odkrili so zmanjšanje števila celic iz nekaterih po Gramu negativnih in po Gramu pozitivnih bakterijskih skupin, v celoti pa se je razmerje med po Gramu negativnimi in pozitivnimi povečalo v korist slednjih. Odgovor na povišane količine plazemskega endotoksina so bile povišane koncentracije glukoze, nato inzulina, kar je na koncu imelo za posledico kopičenje maščob in razvoj metabolnih bolezni. V nadaljevanju so pokazali, da krma z visokim deležem maščob zmanjša število predstavnikov iz rodu *Bifidobacterium*, ter iz skupin *Eubacterium rectale* – *Clostridium coccoides*, kar naj bi povzročilo tudi večjo prepustnost prebavnega epitela, posledica česar je več LPS v krvi. Ugotovili so tudi, da kronična endotoksimija povečuje pojavnost debelosti

(Slika 1), inzulinske rezistence in diabetesa. V mehanizmu v ozadju naj bi bili vpleteni vnetni citokini, saj povišane vrednosti LPS v krvi povečujejo njihovo ekspresijo preko receptorja za LPS CD14. Povišane ravni vnetnih faktorjev fosforilirajo inzulinske receptorje, jih s tem inaktivirajo, posledica pa je razvoj inzulinske rezistence. Ti rezultati kažejo, da lahko do sprememb v sestavi mikrobne združbe pride ob spremembi deleža maščob v krmi ter da te spremembe vplivajo na pojavnost metabolnih bolezni (Cani in sod., 2008).

Opisane raziskave strukture in funkcije prebavne mikrobiote podpirajo idejo, da bi lahko s spremembo mikrobne združbe v prebavilih vplivali na shranjevanje maščob v telesu in posledično na izboljšanje ITM in zdravja. Uspešno zdravljenje prekomerne debelosti ne more potekati brez sprememb v načinu prehranjevanja ter zadostne telesne aktivnosti, kljub temu pa bi lahko modifikacije prebavne mikrobiote proces zdravljenja lajšale. Kažejo se možnosti uporabe antibiotikov, prebiotikov in probiotikov pri ciljanem spreminjanju sestave prebavne mikrobiote. Uspešnost takšnih zdravljenj je bila zaenkrat pokazana pri podganah (Brugman in sod., 2006), miših (Cani in sod., 2007b) in tudi ljudeh. Pri podganah je uporaba antibiotikov zmanjšala pojavnost diabetesa, z anti-biotični zdravljenji ljudi pa so z zmanjšanjem po Gramu



Slika 1: Shematski prikaz doslej odkritih mehanizmov, preko katerih prebavna mikrobiota vpliva na razvoj debelosti. AMPK, adenosin-monofosfat proteinska kinaza; ChREBP, transkripcijski regulator glukoznega in lipidnega metabolizma; Fiaf, adipocitni hormon; LPS, lipopolisaharid; PGC-1 α , transkripcijski kofaktor; SREBP-1, transkripcijski faktor.

Figure 1: Schematic view of so far identified mechanisms through which digestive microbiota affects the development of obesity. AMPK, adenosin-monophosphate protein kinase; ChREBP, carbohydrate-responsive element-binding protein; Fiaf, fasting-induced adipocyte factor; LPS, lipopolisaccharide; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; SREBP-1, sterol response element-binding protein type 1.

negativnih bakterij v prebavilu uspeli znižati ravni LPS in nenormalnega zadrževanja lipidov v jetrnih celicah (Di-Baise, 2008).

Razumevanje interakcij prebavne mikrobiote in gostitelja pri razvoju debelosti se je šele začelo. Odnosa med prebavno mikrobioto in spremembo v telesni masi gostitelja še ne razumemo, saj še ni pojasnjena vloga različnih deležev firmikutov in bakteroidet pri debelih oziroma suhih živalih in ljudeh. Predvsem se kaže potreba po nedvoumnih dokazih, da so spremembe prebavne mikrobiote pri debelih/suhih osebkih posledica oziroma vzrok za debelost/suhost. Nejasni so tudi mehanizmi, po katerih pride do sprememb v prebavni mikrobioti in celoten nabor dejavnikov, ki na to vplivajo. Prebavno mikrobioto ljudi je trenutno mogoče slediti v fecesu, pregled mikrobne združbe iz različnih delov prebavil poskusnih živali pa kaže, da se njena sestava razlikuje glede na lokacijo v črevesu (Camp in sod., 2009). Potrebne bodo dodatne študije strukture mikrobne združbe v celotnem prebavilu ljudi, preden bomo lahko z večjo zanesljivostjo povezali strukturo prebavne mikrobiote z debelostjo. Prav tako bo potrebno ovrednotiti dejanski vpliv prebavne mikrobiote pri pridobivanju energije iz hrane in se prepričati, da te spremembe občutno vplivajo na telesno maso in razvoj metabolnih bolezni.

7 POVZETEK

Prekomerno povečanje telesne mase in kopičenje maščobnih tkiv večinoma dojemamo kot posledico preobilnega hranjenja in nezadostne telesne aktivnosti, kar vodi v razvoj debelosti ter z njo povezanih bolezni, kot so bolezni srca in ožilja, diabetes in rakava obolenja. Zaradi povečane incidence debelosti, ki jo je svetovna znanstvena organizacija prepoznala kot epidemijo, v zadnjem desetletju intenzivneje preučujejo genetske in okoljske dejavnike, ki vplivajo na regulacijo energije. Med dejavnike, ki so vpleteni v razvoj debelosti, se je uvrstila tudi prebavna mikrobiota. Prebavni mikroorganizmi so udeleženi tako pri vnosu, kot tudi porabi in shranjevanju energije, kaže pa se tudi povezava med strukturo mikrobne združbe in gostiteljevim fenotipom. V prebavnem traktu sesalcev večina bakterij pripada debloma *Firmicutes* in *Bacteroidetes*. Pri debelih osebkih se kaže značilno povečano število firmikutov v primerjavi s suhimi, povečanje njihovega relativnega števila pa je značilno tudi ob uživanju visoko kalorične hrane. Vloga različnih deležev firmikutov in bakteroidet pri debelih oziroma suhih živalih in ljudeh še ni pojasnjena, kaže pa se, da ima prebavna mikrobiota vlogo pri pridobivanju energije iz hrane. Primerjave gnobiotskih sterilnih in običajnih miši so pokazale, da prebavna mikrobiota povečuje izplen energije iz

hrane, prebavna mikrobiota debelih živali pa je pri tem še uspešnejša. Študije primerjalne metagenomike so v nadaljevanju pri debelih osebkih odkrile povišane deleže genov, ki kodirajo encime za razgradnjo kompleksnih polisaharidov, in predlagale, da je mikrobiom debelih gostiteljev sposoben pridobiti več energije iz enake količine hrane. Sprememba v združbi prebavne mikrobiote, do katere pride ob spremembi deleža maščob v hrani, naj bi vplivala tudi na pojavnost metabolnih bolezni, in sicer preko vnetnega odziva na povečan vnos bakterijskega LPS. Dosedanje ugotovitve obetajo povsem nov pogled na funkcijo prebavil in razširitev definicije zdravja na interakcije med gostiteljem in njegovo prebavno mikrobioto. Kljub temu pa so potrebne dodatne študije, ki bodo dokončno pojasnile mehanizme teh interakcij, vzroke, zaradi katerih pride do sprememb v prebavni mikrobioti, ter povezave teh sprememb z debelostjo.

8 CONCLUSION

Excessive weight gain and accumulation of body fat is largely perceived as a result of excessive consumption and insufficient physical activity, leading to the development of obesity and with obesity related disorders such as cardiovascular disease, diabetes and cancer. Increasing incidence of obesity was recognized as an obesity epidemic. Therefore, over the last decade environmental and genetic factors that affect energy regulation have been intensively studied. Among factors involved in the development of obesity, the gut microbiota has been also recognized as important. Gastrointestinal microorganisms are involved both in harvest as well as consumption of energy, and a link between microbial community structure and host genotype has also been shown. Most of the bacteria in the gut of mammals belong to two phyla, the *Bacteroidetes* and the *Firmicutes*. In obese animals and humans, a significant increase in the number of the *Firmicutes* was discovered and their relative increase was also characteristic for caloric food intake. The mechanism responsible for changes in numbers of *Firmicutes* and *Bacteroidetes* has not yet been elucidated, but it has been suggested that gut microbiota plays a role in obtaining energy from food. Studies on germ-free animal models have shown that the gut microbiota increases recovery of energy from food and that the gut microbiota from obese animals can harvest energy even more efficient. Comparative metagenomic studies revealed an increased proportion of genes coding for enzymes that are involved in degradation of complex polysaccharides and it has been suggested that obese microbiome is able to obtain more energy from the same amount of food. Changes in the gut microbial community that occur with

the change of the proportion of fats in the diet are supposed to also affect the incidence of metabolic disorders via an increased inflammatory response to bacterial LPS. Up to date, these findings promise a completely new perspective on the function of the gastrointestinal tract and expand our definition of health on interactions between the host and the gut microbial symbionts. However, further studies are needed to unequivocally clarify the mechanisms of these interactions, to identify reasons for changes in the structure of gut microbiota and to link these changes to obesity.

9 VIRI

- Avbelj M., Saje-Hribar N., Seher-Zupančič M., Brcar P., Kotnik P., Iršič A., Bratanič N., Kržišnik C., Battelino T. 2005. Prevalenca čezmerne prehranjenosti in debelosti med pet let starimi otroki in 15 oziroma 16 let starimi mladostniki in mladostniki v Sloveniji. *Zdravniški Vestnik*, 74: 753–9
- Bäckhed F., Ding H., Wang T., Hooper L.V., Koh G.Y., Nagy A., Semenkovich C.F., Gordon J.I. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U. S. A.*, 101: 15718–15723
- Bäckhed F., Ley R.E., Sonnenburg J.L., Peterson D.A., Gordon J.I. 2005. Host-bacterial mutualism in the human intestine. *Science*, 307: 1915–1920
- Bäckhed F., Manchester J.K., Semenkovich C.F., Gordon J.I. 2007. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. U. S. A.*, 104: 979–984
- Bates S.H., Myers M.G. 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. *Trends in Endocrinology and Metabolism*, 14: 447–452
- Berg R.D. 1996. The indigenous gastrointestinal microflora. *Trends in Microbiology*, 4: 430–435
- Bjursell M.K., Martens E.C., Gordon J.I. 2006. Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, *Bacteroides thetaiotaomicron*, to the suckling period. *Journal of Biological Chemistry*, 281: 36269–36279
- Brugman S., Klatter F.A., Visser J.T., Wildeboer-Veloo A.C., Harmsen H.J., Rozing J., Bos N.A. 2006. Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? *Diabetologia*, 49: 2105–2108
- Calle E.E., Thun M.J. 2004. Obesity and cancer. *Oncogene*, 23: 6365–6378
- Camp J.G., Kanther M., Semova I., Rawls J.F. 2009. Patterns and scales in gastrointestinal microbial ecology. *Gastroenterology*, 136: 1989–2002
- Cani P.D., Neyrinck A.M., Maton N., Delzenne N.M. 2005. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obesity Research*, 13: 1000–1007
- Cani P.D., Delzenne N.M. 2007a. Gut microflora as a target for energy and metabolic homeostasis. *Current Opinion in Clinical Nutrition and Metabolic Care*, 10: 729–734
- Cani P.D., Neyrinck A.M., Fava F. 2007b. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*, 50: 2374–2383
- Cani P.D., Bibiloni R., Knauf C., Neyrinck A.M., Neyrinck A.M., Delzenne N.M., Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 57: 1470–1481
- Cani P.D., Delzenne N.M. 2009. The role of the gut microbiota in energy metabolism and metabolic disease. *Current Pharmaceutical Design*, 15: 1546–1558
- Collado M.C., Isolauri E., Laitinen K., Salminen S. 2008. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *American Journal of Clinical Nutrition*, 88: 894–899
- Couturier C., Sarkis C., Seron K., Belouzard S., Chen P., Lennain A., Corset L., Dam J., Vauthier V., Dubartt A., Mallet J., Froguel P., Rouille Y., Jockers R. 2007. Silencing of OB-RGRP in mouse hypothalamic arcuate nucleus increases leptin receptor signaling and prevents diet-induced obesity. *Proc. Natl. Acad. Sci. U. S. A.*, 104: 19476–19481
- Dethlefsen L., Huse S., Sogin M.L., Relman D.A. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology*, 6: 2383–2400
- Dethlefsen L., McFall-Ngai M., Relman D.A. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*, 449: 811–818
- DiBaise J.K., Zhang H., Crowell M.D., Krajmalnik-Brown R., Decker G.A., Rittmann B.E. 2008. Gut microbiota and its possible relationship with obesity. *Mayo Clinic Proceedings*, 83: 460–469
- Dore J. 2009. A microbiomics view of the human intestinal microbiota. V: FEMS 3rd Congress of European Microbiologists. Danone Satellite Symposium: Microbes for Health.
- Eckburg P.B., Bik E.M., Bernstein C.N., Purdom E., Dethlefsen L., Sargent M., Gill S.R., Nelson K.E., Relman D.A. 2005. Diversity of the human intestinal microbial flora. *Science*, 308: 1635–1638
- Gill S.R., Pop M., Deboy R.T., Eckburg P.B., Turnbaugh P.J., Samuel B.S., Gordon J.I., Relman D.A., Fraser-Liggett C.M., Nelson K.E. 2006. Metagenomic analysis of the human distal gut microbiome. *Science*, 312: 1355–1359
- Guo X., Xia X., Tang R., Zhou J., Zhao H., Wang K. 2008. Development of a real-time PCR method for *Firmicutes* and *Bacteroidetes* in faeces and its application to quantify intestinal population of obese and lean pigs. *Letters in Applied Microbiology*, 47: 367–373
- Kalliomaki M., Collado M.C., Salminen S., Isolauri E. 2008. Early differences in fecal microbiota composition in children may predict overweight. *American Journal of Clinical Nutrition*, 87: 534–538
- Leamon J.H., Lee W.L., Tartaro K.R., Lanza J.R., Sarkis G.J., deWinter A.D., Berka J., Lohman K.L. 2003. A massively parallel PicoTiterPlate (TM) based platform for discrete

- picoliter-scale polymerase chain reactions. *Electrophoresis*, 24: 3769–3777
- Ley R.E., Bäckhed F., Turnbaugh P., Lozupone C.A., Knight R.D., Gordon J.I. 2005. Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U. S. A.*, 102: 11070–11075
- Ley R.E., Turnbaugh P.J., Klein S., Gordon J.I. 2006a. Microbial ecology: human gut microbes associated with obesity. *Nature*, 444: 1022–1023
- Ley R.E., Peterson D.A., Gordon J.I. 2006b. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124: 837–848
- Mahowald M.A., Rey F.E., Seedorf H., Turnbaugh P.J., Fulton R.S., Wollam A., Shah N., Wang C., Magrini V., Wilson R.K., Cantarel B.L., Coutinho P.M., Henrissat B., Crock L.W., Russell A., Verberkmoes N.C., Hettich R.L., Gordon J.I. 2009. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc. Natl. Acad. Sci. U. S. A.*, 106: 5859–5864
- Margulies M., Egholm M., Altman W.E., Attiya S., Bader J.S., Bemben L.A., Berka J., Braverman M.S., Chen Y.J., Chen Z.T., Dewell S.B., Du L., Fierro J.M., Gomes X.V., Godwin B.C., He W., Helgesen S., Ho C.H., Irzyk G.P., Jando S.C., Alenquer M.L.L., Jarvie T.P., Jirage K.B., Kim J.B., Knight J.R., Lanza J.R., Leamon J.H., Lefkowitz S.M., Lei M., Li J., Lohman K.L., Lu H., Makhijani V.B., McDade K.E., McKenna M.P., Myers E.W., Nickerson E., Nobile J.R., Plant R., Puc B.P., Ronan M.T., Roth G.T., Sarkis G.J., Simons J.F., Simpson J.W., Srinivasan M., Tartaro K.R., Tomasz A., Vogt K.A., Volkmer G.A., Wang S.H., Wang Y., Weiner M.P., Yu P.G., Begley R.F., Rothberg J.M. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, 437: 376–380
- Mariat D., Firmesse O., Levenez F., Guimaraes V.D., Sokol H., Dore J., Corthier G., Furet J.P. 2009. The *Firmicutes/Bacteroidetes* ratio of the human microbiota changes with age. *BMC Microbiology*, 9: 123–128
- Ministrstvo za zdravje RS. 2007. Strategija Vlade Republike Slovenije na področju telesne (gibalne) dejavnosti za krepitev zdravja od 2007 do 2012. Ljubljana: 56 str.
- Mozes S., Bujnakova D., Sefcikova Z., Kmet V. 2008. Intestinal microflora and obesity in rats. *Folia Microbiologica*, 53: 225–228
- Mueller S., Saunier K., Hanisch C., Norin E., Alm L., Midtvedt T., Cresci A., Silvi S., Orpianesi C., Verdenelli M.C., Clavel T., Koebnick C., Zunft H.J., Dore J., Blaut M. 2006. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Applied and Environmental Microbiology*, 72: 1027–1033
- Nadal I., Santacruz A., Marcos A., Warnberg J., Garagorri M., Moreno L.A., Martin-Matillas M., Campoy C., Marti A., Moleres A., Delgado M., Veiga O.L., Garcia-Fuentes M., Redondo C.G., Sanz Y. 2009. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *International Journal of Obesity*, 33: 758–767
- O'Rahilly S. 2002. Insights into obesity and insulin resistance from the study of extreme human phenotypes. *European Journal of Endocrinology*, 147: 435–441
- Palmer C., Bik E.M., DiGiulio D.B., Relman D.A., Brown P.O. 2007. Development of the human infant intestinal microbiota. *PLoS Biology*, 5: 1556–1573
- Rankinen T., Perusse L., Weisnagel S.J., Snyder E.E., Chagnon Y.C., Bouchard C. 2001. The human obesity gene map: the 2001 update. *Obesity Research*, 10: 196–243
- Rawls J.F., Mahowald M.A., Ley R.E., Gordon J.I. 2006. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell*, 127: 423–433
- Samuel B.S., Gordon J.I. 2006. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. *Proc. Natl. Acad. Sci. U. S. A.*, 103: 10011–10016
- Samuel B.S., Hansen E.E., Manchester J.K., Coutinho P.M., Henrissat B., Fulton R., Latreille P., Kim K., Wilson R.K., Gordon J.I. 2007. Genomic and metabolic adaptations of *Methanobrevibacter smithii* to the human gut. *Proc. Natl. Acad. Sci. U. S. A.*, 104: 10643–10648
- Tringe S.G., von Mering C., Kobayashi A., Salamov A.A., Chen K., Chang H.W., Podar M., Short J.M., Mathur E.J., Detter J.C., Bork P., Hugenholtz P., Rubin E.M. 2005. Comparative metagenomics of microbial communities. *Science*, 308: 554–557
- Turnbaugh P.J., Ley R.E., Mahowald M.A., Magrini V., Mardis E.R., Gordon J.I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444: 1027–1031
- Turnbaugh P.J., Ley R.E., Hamady M., Fraser-Liggett C.M., Knight R., Gordon J.I. 2007. The Human Microbiome Project. *Nature*, 449: 804–810
- Turnbaugh P.J., Hamady M., Yatsunenko T., Cantarel B.L., Duncan A., Ley R.E., Sogin M.L., Jones W.J., Roe B.A., Affourtit J.P., Egholm M., Henrissat B., Heath A.C., Knight R., Gordon J.I. 2009. A core gut microbiome in obese and lean twins. *Nature*, 457: 480–484
- Zoetendal E.G., Akkermans A.D.L., Akkermans-van Vliet W.M., de Visser J.A.G.M., de Vos W.M. 2001. The host genotype affects the bacterial community in the human gastrointestinal tract. *Microbial Ecology in Health and Disease*, 13: 129–134
- Wellen K.E., Hotamisligil G.S. 2005. Inflammation, stress, and diabetes. *Journal of Clinical Investigation*, 115: 1111–1119
- Yang W.J., Kelly T., He J. 2007. Genetic epidemiology of obesity. *Epidemiologic Reviews*, 29: 49–61

PREVALENCE OF COAGULASE POSITIVE PATHOGENIC *Staphylococcus aureus* IN MILK AND MILK PRODUCTS COLLECTED FROM UNORGANIZED SECTOR OF AGRA

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Prevalence of coagulase positive pathogenic Staphylococcus aureus in milk and milk products collected from unorganized sector of Agra

The present study deals with the sampling, isolation and biochemical characterization of *Staphylococcus aureus* from raw milk of cow and buffalo, and milk products (khoa sweets and cottage cheese) collected from different unorganized sectors of Agra city, India. Samples of raw milk from cow, cottage cheese and khoa sweets indicated that *S.aureus* contamination was higher in east of Agra (Ram Bagh), whereas samples of raw milk from buffalo, collected from the west of Agra (Sikandra), showed higher *S.aureus* contamination. Least contamination was observed in samples of khoa sweets and cottage cheese collected from west of Agra (Sikandra) and in samples of cow and buffalo milk collected from North of Agra (Dayalbagh) and east of Agra (Ram Bagh), respectively. The incidence of high contamination in raw milk and milk products suggests that protective measures are inadequate and need to be enforced to avoid potential threat to public health.

Key words: milk / milk products / microbiology / *Staphylococcus aureus* / isolation / biochemical characterization

Pogostnost okužb mleka in mlečnih proizvodov s patogenimi sevi bakterije Streptococcus aureus s koagulazno aktivnostjo na območju neorganizirane proizvodnje v okolici Agra (Indija)

Prispevek opisuje vzorčenje, osamitev in biokemijsko karakterizacijo bakterije *Staphylococcus aureus* iz surovega mleka krav in bivolov ter mlečnih proizvodov (khoa in skuta) na neorganiziranem proizvodnem območju mesta Agra v Indiji. Vzorci surovega kravjega mleka, skute in khoe kažejo, da je bila kontaminacija s *S. aureus* močnejša na vzhodu Agra (Ram Bagh), medtem ko so bili vzorci surovega mleka bivolov bolj kontaminirani na območju zahodnega dela Agra (Sikandra). Najnižjo raven kontaminacije smo opazovali v vzorcih khoe in skute z zahodnega dela Agra (Sikandra) in v vzorcih kravjega in bivoljega mleka s severnega (Dayalbagh) in vzhodnega dela Agra (Ram Bagh). Visoka stopnja kontaminacije mleka in mlečnih proizvodov kaže, da zaščitni ukrepi za preprečevanje kontaminacij niso ustrezni in jih je nujno treba izboljšati, da bi se izognili ogrožanju javnega zdravja.

Ključne besede: mleko / mlečni izdelki / mikrobiologija / *Staphylococcus aureus* / izolacija / biokemična karakterizacija

1 INTRODUCTION

Milk and milk products are home to complex microbial ecosystems; these are responsible for the broad variations in taste, aroma and texture of milk and milk products. Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment. Milk and milk products like cottage cheese and khoa sweets are widely consumed since ancient times and its market demand is continuous throughout the world. The occurrence of pathogenic

bacteria in these milk and milk products can cause severe health hazards to people. *S.aureus* is one of those bacteria that can cause minor skin infections (pimples, boils, cellulites, toxic shock syndrome, impetigo and abscesses) as well as life threatening diseases (pneumonia, meningitis, endocarditis and septicemia) (Soomro *et al.*, 2003).

S.aureus 0.5–1.59 μ m in diameter are spherical Gram positive, non motile, non-spore forming facultative anaerobes which ferment most of the sugars except raffinose and salicin producing lactic acid during fermentation. They are catalase and coagulase positive and flour-

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ish between a pH of 7.4–7.6. They exist in air, dust water, sewage, meat and meat products, poultry and egg products, salad such as tuna, chicken, potato and macaroni, bakery products such as cream filled pastries, cream pies, chocolates éclairs, and sandwich fillings and in milk and milk products. Pathogenesis of *S.aureus* is due to repertoire toxins, exoenzyme adhesions and immune modulating protein that it produces. 20–30% of healthy people may carry this bacterium on their skin surface and nasal passage (MedicineNet.com). It causes a variety of superlative infections by producing leukocidin, a toxin that destroys the white blood cells and leads to the formation of pus and toxinos in humans (Todar, 2005). The presence of *S.aureus* in food causes food poisoning by releasing enterotoxins into the food and it can also cause Toxic Shock Syndrome by release of super antigens into the blood stream (Todar, 2005).

Staphylococci possess protein receptors for many mammalian proteins such as fibronectin, fibrinogen and IgG, which facilitate Staphylococcal adhesion to host cell and tissue (Todar, 2005). It can grow at a temperature range from 15 to 45 °C and at NaCl concentration as high as 15 percent (Wenzel and Perl, 1995). About 50% strains of this organism are able to produce enterotoxin associated with food poisoning (Pyne and Wood, 1974). On heating at normal cooking temperature, the bacteria may be killed but the toxins remain (Prescott *et al.*, 2002). As little as 1.0 9g toxin in contaminated food produces symptoms of illness. This level of the toxin has been found at 105 cells /g of food; this toxin cannot be denatured after boiling (Ananthanarayana *et al.*, 2001). It can also grow at high salt which is generally 15% higher than normal. *S.aureus* also expresses certain virulence factors and due to these virulent determinants, it is tenacious, potentially destructive and shows increasing resistance to antimicrobial agents. The coagulase test is a standard criteria for the identification of *S.aureus* (Burriel, 1998).

Pathogenicity of *S.aureus* is due to the membrane active substances i.e. cytolytic toxins, consisting of four haemolysins and a leukocidin. This genus may have alpha, beta, gamma and delta haemolysin and the pathogenic members of species *aureus* display beta haemolysis (Presscott, 2002). The present study attempts to assess the prevalence and pathogenicity of *S.aureus* in milk (cow and buffalo) and milk products (khoa sweets and cottage cheese) collected from different unorganized sectors of Agra city, because unorganized sectors consist of all private enterprises whose activities are not regulated under legal provision. In Agra, there are many unorganized areas where milk and milk products are produced by traditional methods and these milk and milk products are usually consumed locally.

2 MATERIALS AND METHODS

Standard strains: Standard strain of *S.aureus* (MTCC-3103) was procured from MTCC Chandigarh. All the isolates were confirmed through biochemical tests by comparing with the results of standard strains. **Collection of samples:** Different samples of milk and milk products were collected from different regions of Agra city including Dayalbagh, Sikandra, Ram Bagh and Sultan Pura and examined for the presence of *S.aureus*. Raw milk samples were collected into sterile test tubes from individual cows and buffaloes while the milk products were collected aseptically, transferred to sterile plastic bags and directly transported to the laboratory under cold conditions and analyzed within 24 hrs.

Biochemical analysis: A portion (10 g or 10 ml) from the centre of each sample was extracted aseptically and homogenized with 90 ml sterile enrichment broth peptone water and incubated at 37 °C for 24 hrs, for further biochemical analysis. *S.aureus* was isolated by using the Baird Parker's (1962) technique. Enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hrs. Appearance of jet black colonies surrounded by a white halo were considered to be presumptive *S.aureus* and were further analyzed by Gram's reaction and biochemical tests (Table 1).

Confirmation of the genus, *Staphylococcus* was done by Gram staining and biochemical tests-catalase test, oxidase test, indole, methyl red, Voges-Proskauer

Table 1: Biochemical characterization of *S.aureus* strains
Preglednica 1: Biokemijska karakterizacija sevov *S. aureus*

Reaction		<i>S.aureus</i>
Catalase		+
Oxidase		–
Indole Production		–
Nitrate Reduction		+
Methyl Red		+
Voges-Proskauer		+
Haemolysis		+
Coagulase		+
Acid from shugar	Glucose	+
	Mannitol	+
	Maltose	+
	Lactose	+
	Raffinose	–
	Sucrose	+

+ = Positive reaction / Pozitivna reakcija;
– = Negative reaction / Negativna reakcija

test, nitrate reduction, acid from different sugars while the species, *S.aureus* was confirmed by the coagulase test (Monica 1991). Pathogenicity of *S.aureus* was confirmed by beta haemolysis on Sheep Blood Agar (S.B.A.) following the method of Cruickshank (1970).

3 RESULTS

Samples were collected in different regions of Agra city. Table 2 depicts the sampling data, which showed the percentage of prevalence of pathogenic *S.aureus* isolated from the various samples of cow milk, buffalo milk, khoa sweets and cottage cheese collected from different unorganized sectors of Agra city. A total of 64 cow milk samples, 52 buffalo milk samples, 71 cottage cheese samples and 69 khoa sweet samples were analyzed for the isolation of coagulase positive pathogenic *S.aureus*, collected from different sites of Agra city including Ram Bagh (East), Sikandra (West), Dayalbagh (North) and Sultan Pura (South) (Fig. 1).

Cow milk samples: Out of 64 cow milk samples, 42 isolates were confirmed as coagulase positive *S.aureus* and from these 25 were confirmed as pathogenic *S.aureus*.

Buffalo milk samples: Out of 52 buffalo milk samples, 33 isolates were confirmed as coagulase positive *S.aureus* of which 15 were confirmed as pathogenic *S.aureus*.

Cottage cheese samples: Out of 71 cottage cheese samples, 43 isolates were confirmed as coagulase positive *S.aureus* of which 9 were confirmed as pathogenic *S.aureus*.

Khoa sweets samples: Out of 64 khoa sweets samples, 47 isolates were confirmed as coagulase positive *S.aureus* and from these 13 were confirmed as pathogenic *S.aureus*.

Data analysis showed that *S.aureus* contamination was higher in samples of cow milk, cottage cheese and khoa sweets collected from the eastern part (Ram Bagh) of Agra city. Buffalo milk samples collected from the in western part (Sikandra) of Agra city showed a higher contamination. Khoa and cottage cheese samples from

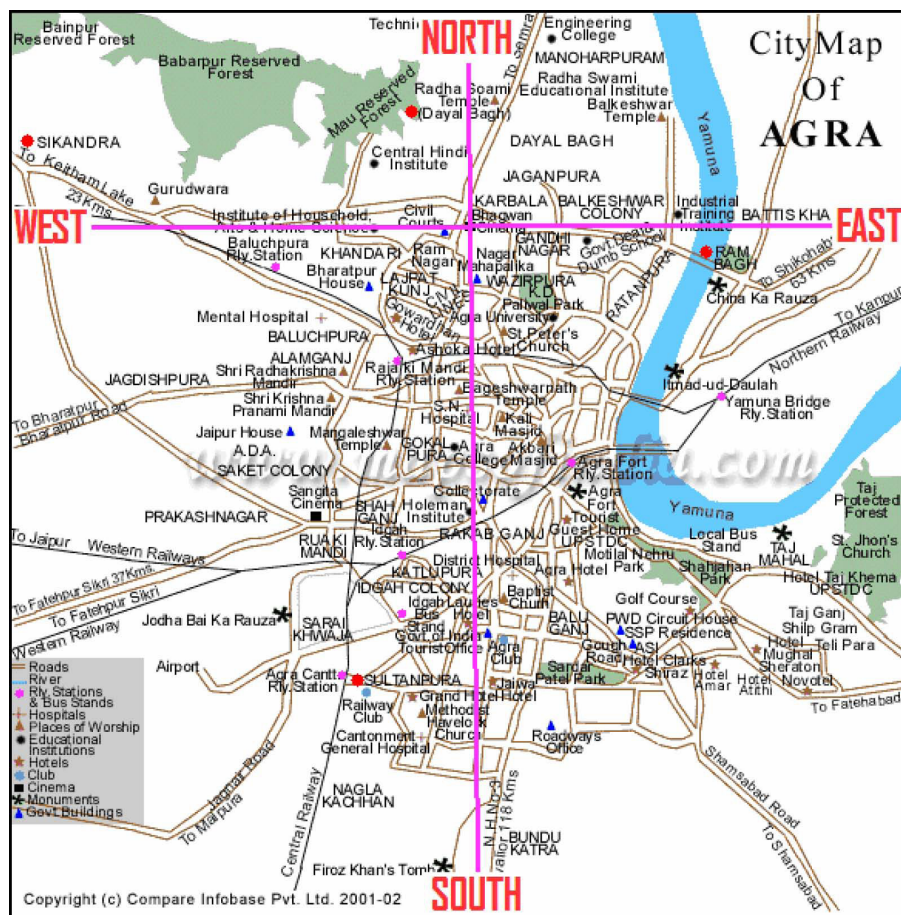


Figure 1: Map of Agra showing different sites of sampling.
Slika 1: Zemljevid z mesti vzorčenja.



Figure 2: Plate showing beta haemolysis on 5% sheep blood agar.
Slika 2: Test hemolizne aktivnosti na 5 % krvnem agarju iz ovčje krvi

Sikandra showed the least contamination. Raw milk samples both from cow and buffalo procured from Day-albagh and Ram Bagh were least contaminated. (Fig. 3, Table 2)

4 DISCUSSION

Milk is normally sterile in the udder of the cow and buffalo provided they do not suffer from mastitis (udder infection). If they have mastitis, a large number of generally Gram positive bacteria such as *Streptococcus* and *Staphylococcus* spp. may be present in milk when it leaves the udder (Holm and Jespersen, 2003). Further, milk may be often contaminated on its way to the bulk tank as rarely the hygienic procedure is followed by the local dairy. Khoa is made from the thickening of the milk at high temperature, which expectedly kills all the bacteria incriminated during handling, storage and transportation.

Sweets made from khoa may be kept for days or weeks and may then be contaminated with the bacteria. On the other hand, cottage cheese in industries is manufactured by automated process, so the chances of contamination are fewer. In villages and unorganized sectors cheese is prepared by traditional methods that increases the probability of contamination during the processing and may cause the transfer of undesirable microorganisms in the end products. Proper hygiene and safe food practices are essential to prevent the presence of organisms such as *Listeria monocytogenes* and *S.aureus* in the milk products.

Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udders, milking equipments, cooking temperature, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram-negative bacteria in the bulk tank milk (Vasavada, 1988; Bonfoh *et al.*, 2003).

These parameters play an important role in determining the characteristics of milk and milk products, which are one of the major sources of protein in a vegetarian's diet. If these foods are contaminated with *S.aureus*, they mean a serious health problem. Raw milk is used to collect cream and this cream is further used to produce various value added products, however, this cream may serve as a major vehicle for transmission of pathogens. The analyzed raw milk samples both from buffalo and cow were contaminated with pathogenic *S.aureus*, therefore, the presence of pathogenic bacteria in milk becomes a major concern to public health, its consumption, or use in the production of dairy products such as sweets, cheese, butter, cream and ice-cream, without pasteurization or subjection to a high temperature for sufficient time may lead to various health problems among masses. As Agra is the city where innumerable tourists flock from different parts of India as well as the world, therefore it can be a serious problem to the local consumers and the

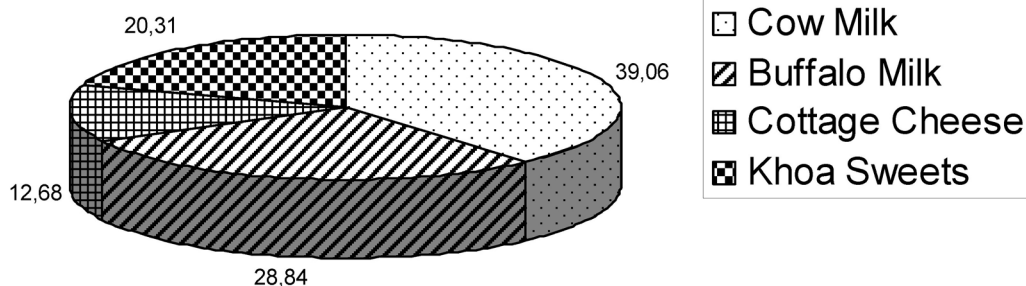


Figure 3: Percentage of prevalence of *S.aureus* in milk and milk products.
Slika 3: Pogostnost pojavljanja *S. aureus* v mleku in mlečnih izdelkih.

Table 2: Percentage of incidence of *S.aureus* in different areas of Agra city**Preglednica 2:** Pogostnost pojavljanja *S. aureus* na različnih območjih mesta Agra

Source	Area	Total No. of isolates analyzed	No. of isolates confirmed as <i>S.aureus</i>	No. of isolates confirmed to be pathogenic <i>S.aureus</i>	Percentage of isolates confirmed to be pathogenic
Cow Milk	Dayalbagh	20	10	2	10
	Sikandra	8	7	4	50
	Sultan Pura	24	21	12	50
	Ram Bagh	12	11	7	58
Buffalo Milk	Dayalbagh	20	12	5	25
	Sikandra	4	4	4	100
	Sultan Pura	16	8	4	25
	Ram Bagh	12	9	2	16
Cottage cheese	Dayalbagh	21	9	2	9.5
	Sikandra	20	12	0	0
	Sultan Pura	19	13	4	21
	Ram Bagh	11	9	3	27
Khoa sweets	Dayalbagh	13	8	2	15.3
	Sikandra	8	3	0	0
	Sultan Pura	20	14	2	10
	Ram Bagh	28	22	9	32

tourists when the food is incriminated with the pathogenic *S.aureus*.

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6 REFERENCES

- Ananthanarayan R., Panikaran, C.K.J. 2001. Diagnostic value of mannitol for sugar fermentation in *S.aureus*. Textbook of Microbiology, 6: 178–186
- Baird-Parker A. 1962. An improved diagnostic and selective medium for isolating coagulase positive *Staphylococci*. Journal of applied bacteriology, 25: 12–19
- Bonfoh B. Wasem A., Traore A.N., Fane A., Spillmann H., Simbe C. F., Alfaroukh I.O., Nicolet J., Farah Z., Zinsstag J. 2003. Microbiological quality of cow's milk taken at different intervals from the udder to the selling point in Bamako (Mali). Food Control, 14: 495–500
- Burriel A.R. 1998. Isolation of Coagulase positive *Staphylococci* from the milk and milk and environment of sheep. J. Dairy Sci., 65: 139–142
- Cruickshank R. 1965. Medical Microbiology. Cruickshank (ed). London, ELBS and E and S Livingstone: 130 p.
- Holm C., Jespersen L. 2003. A flow- cytometric Gram-Staining technique for milk associated bacteria. Applied and Environmental Microbiology, 69: 2857–2863
- Medicine- *Staphylococcus aureus* infection. 2009. www.MedicineNet.com
- Monica, C. 1991. Medical Laboratory manual for Tropical countries. VOL 11. ELBS: 60–63.
- Payne D.N., Wood J.M. 1974. The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from food. J Applied Bacteriol., 319–325
- Presscott L.M., Harley J.P., Klein D.A. 2002. Text book of Microbiology. Brown Publishers. 5th ed.: 441–42
- Soomro A.H., Arain M.A., Khaskheli M., Bhutto B. 2003. Isolation of *Staphylococcus aureus* from milk products sold at sweet meat shops of Hyderabad. Online Journal of Biological Sciences, 3, 1: 91–94
- Todar K. 2005. Pathogenesis of *S.aureus*. Original text book by Kenneth Todar. University of Wisconsin
- Vasavada P.C. 1988. Pathogenic bacteria in milk-A review. J. Dairy Sci., 71: 2809–2816
- Wenzel R.P., Perl T.M. 1995. The significance of nasal carriage of *S.aureus* and the incidence of prostoperative wound infection. J. Hosp Infect., 31: 13–24 [Medline]

VLOGA MATIČNIH CELIC PRI NASTANKU RAKA MLEČNE ŽLEZE

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Vloga matičnih celic pri nastanku raka mlečne žleze

Rak mlečne žleze je najpogostejša oblika raka pri ženskah, zelo pogost pa je tudi pri nekaterih vrstah domačih živali (psi, mačke). Vse najnovejše raziskave s področja biologije mlečne žleze podpirajo hipotezo rakave matične celice, po kateri naj bi tumor izviral iz matične celice mlečne žleze ali njenih zgodnjih potomk. Tumorji nastanejo zaradi napake v uravnavanju sicer natančno kontroliranega procesa samoobnavljanja matičnih celic, kar je njihova temeljna lastnost. Tak tumor vsebuje subpopulacijo celic z lastnostmi matičnih celic, ki omogočajo razvoj in diferenciacijo tumorja ter prispevajo k celični heterogenosti. Vlogo rakavih matičnih celic v rakavem tkivu lahko primerjamo z vlogo normalnih matičnih celic v zdravem tkivu. Njihov obstoj je bil sprva dokazan pri krvnem raku, kasneje pa še pri malignih spremembah kompaktnih tkiv: možgan, prostate, debelega črevesa, trebušne slinavke in mlečne žleze. Identifikacija matičnih celic v zdravem in tumorskem tkivu mlečne žleze je razkrila vzroke za molekularno kompleksnost raka mlečne žleze pri ljudeh in živalih. Hipoteza rakave matične celice ima pomembno vlogo pri zgodnjem odkrivanju in zdravljenju dedne in sporadične oblike raka dojke pri ženskah. Matične celice z okvarjenim mehanizmom samoobnavljanja so odporne na obsevanje in kemoterapijo in zato predstavljajo osrednjo tarčo pri razvoju novih zdravil in strategij za zdravljenje raka dojke.

Ključne besede: mlečna žleza / matične celice / rak / medicina

The role of breast cancer stem cells in development of breast cancer

Breast cancer is the most common type of cancer in women worldwide but also very common in domestic cats and dogs. Recent research in mammary gland biology has provided support for the cancer stem cell-hypothesis. The main focus of this hypothesis is that tumor originates from mammary stem or progenitor cells as a result of deregulation of the normally precise regulated process of self-renewal, which is their main characteristics. As a result, tumors contain and are driven by a cellular subcomponent that retains key stem-cell properties and enable development and differentiation of the tumor tissue, which results in extensive cellular heterogeneity. The role of cancer stem cells in cancer tissues resembles the role of normal stem cells in healthy tissue. Cancer stem cells have been first discovered in hematopoietic and later in solid cancers like brain, prostate, colon, pancreas and breast cancer. Identification of these cells in healthy and tumor breast tissue has helped to elucidate the origin of molecular complexity of breast cancer in humans and animals. The cancer stem-cell hypothesis has important implications for early detection and prevention of hereditary and sporadic form of breast cancer in humans. Aberrant stem cells with deregulated self-renewal mechanism are highly resistant to radiation and chemotherapy and therefore represent a central target in development of more effective drugs and therapies for breast cancer treatment.

Key words: mammary gland / stem cells / cancer / medicine

1 MLEČNA ŽLEZA

Mlečna žleza v življenju samic sesalcev prehaja preko več razvojnih stopenj in sprememb, ki jih uravnavajo številni geni in hormoni. Ob rojstvu je sestavljena iz

manjšega števila mlečnih vodov, ki ležijo v maščobnem vezivnem tkivu in se končajo s terminalnimi brsti. Ti sestojijo iz celic z veliko proliferacijsko aktivnostjo. Nekatere od teh celic imajo lastnosti matičnih celic. Do pubertete mlečna žleza raste sorazmerno z ostalim telesom,

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z začetkom estričnih ciklov pa sistem mlečnih vodov pod vplivom hormonov postane bolj kompleksen. Višek kompleksnosti pomeni laktacija, ki jo žleza doseže takoj po rojstvu potomcev. Razvoj mlečne žleze poteka pod vplivom hormonov. Estrogen povzroči podaljševanje mlečnih vodov, progesteron pa dodatno razvejanje. Terminalni brsti se pretvorijo v alveolarne tvorbe, ki se pod vplivom prolaktina močno razvijajo in izločajo mleko. Razporeditev alveol na mlečnih kanalčkih je med vrstami različna in igra pomembno vlogo pri izoblikovanju končne strukture mlečne žleze. Histološko je zrela žleza sestavljena iz bazalnega in luminalnega sloja celic. Spodnji-bazalni sloj tvorijo na bazalni membrani ležeče mioepitelne celice, ki se pod vplivom hormona oksitocina krčijo in so odgovorne za praznjenje mlečne žleze. Na njih leži luminalni epitelni sloj celic, ki v času laktacije proizvaja mleko. Ko je laktacija končana, sledi involucija, v kateri se z apoptozo in fagocitozo odstranijo odvečne epitelne strukture. Stopnja diferenciranosti mlečne žleze je po nosečnosti večja kot pred njo. Zaradi cikličnega značaja in visoke stopnje proliferacije celic, je mlečna žleza bolj podvržena morebitnim kancerogenim spremembam kot končno diferencirane strukture.

2 MATIČNE CELICE V MLEČNI ŽLEZI

Obstoj matičnih celic je bil dokazan v številnih organizmih in tkivih. Tudi za ciklični razvoj in obnavljanje funkcionalne mlečne žleze po vsaki gravidnosti je nujna prisotnost multipotentnih matičnih celic. Njihov obstoj je že leta 1959 prvi dokazal DeOme s sodelavci. Ugotovil je, da majhni delci tkiva ali terminalni brsti mlečne žleze miši po presaditvi v izpraznjeno prsno maščobno blaznico druge miši, lahko tvorijo novo funkcionalno mlečno žlezo. Študije z metodo inaktivacije X-kromosoma so potrdile obstoj celic, ki lahko tvorijo mioepitelne, kot tudi epitelne celice mlečne žleze pri človeku. Smith s sodelavci je matične celice v mlečni žlezi morfološko identificiral kot nediferencirane, manjše svetle celice, ki ležijo v bazalnem sloju celic (Ponti in sod., 2006). Sledili so poskusi izolacije in gojenja matičnih celic. Welm in sodelavci (2002) so ugotovili, da določene celice mlečne žleze, ki so preko posebnih ABC transporterjev sposobne izločati fluorescentno barvilo Hoechst 33342, lahko po presaditvi v očiščeno prsno maščobno blaznico regenerirajo celotno žlezo. To populacijo celic so imenovali »*stranska populacija*«. (po vzoru SP celic v kostnem mozgu). Sposobnost regeneracije mlečne žleze po presaditvi v NOD/SCID miš je še vedno osnova vseh tehnik izolacije matičnih celic, s katero se učinkovito preveri njihov multipotentni potencial. Alternativna *in vitro* metoda je tridimenzionalni gel, sestavljen iz ustreznih komponent ekstracelularnega

matriksa, obogaten z lamininom in kolagenom. Na takih gelih, ki skušajo posnemati naravne pogoje v žlezi, lahko opazujemo sposobnost diferenciacije domnevnih matičnih celic v različne potomke in tvorbo zametkov terminalnih brstov (LaBarge, 2007). Za izolacijo matičnih celic iz tkiva mlečne žleze se danes uporablja pretočno citometrijo in fluorescentne označevalce (ang.: »FACS – fluorescent activated cell sorting«). Ta metoda temelji na specifičnih površinskih označevalcih, ki jih prepoznajo fluorescentno označena protitelesa in tako omogočijo ločevanje željene populacije celic od drugih celic in na poskusih serijskih presaditev, s katerimi testiramo njihovo multipotentnost. Kombinacija označevalcev, s katerimi so prišli do populacije z ustreznimi lastnostmi je Lin⁻CD29^{hi}CD24⁺ ali Lin⁻CD49f^{hi}CD24⁺, kjer Lin⁻ zajema več označevalcev, ki so značilni za epitelne in krvne celice, ki v tej populaciji niso zaželjene. Za nekatere celice v Lin⁻CD29^{hi}CD24⁺ ali Lin⁻CD49f^{hi}CD24⁺ populaciji so v poskusu večkratnih presaditev dokazali lastnosti matičnih celic (Stingl in sod. 2006). Pomemben cilj raziskav je najti označevalec, značilen samo za matične celice. Za gojenje nediferenciranih matičnih celic in prekursorjskih celic v *in vitro* pogojih danes uporabljamo kulturo »mamosfer«, ki so v gojišču prosto plavajoči kroglasti skupki celic (Vaillant in sod., 2007).

Matične celice mlečne žleze se nahajajo v bazalnem sloju celic. V zgodnjem razvoju so skoncentrirane predvsem v terminalnih brstih, pri zreli žlezi pa so enakomerno razporejene v steni terminanih kanalčkov. Celična zgradba mlečne žleze je predvsem v času intenzivne delitve celic med gravidnostjo zelo heterogena. Razvojna hierarhija teh celic – od matične celice, preko bipotentnih predniških celic in linijsko determiniranih prednic do končno diferenciranih celic – postaja jasna vzporedno z značilnimi markerji, ki so potrebni za njihovo identifikacijo. Med takimi markerji prevladujejo celično specifični keratini. Določitev hierarhičnosti je zanimiva z vidika izvora rakave celice in se zgleduje po celicah krvnega sistema, pri katerih je znano, da se začetna mutacija zgodi v matični celici, nadaljne pa v njenih multipotentnih potomkah. Zelo pomemben dejavnik, ki vpliva na matične celice je tudi njihova niša. To je mikrookolje, ki določa in uravnava njihov razvoj (LaBarge, 2007, Huang in sod., 2006 in Stingl&Caldas, 2007).

3 RAK MLEČNE ŽLEZE

Rak mlečne žleze prizadane kar 10-odstotkov žensk, predvsem iz starejše populacije. Pojavnost te oblike raka med živalskimi vrstami močno variira. Posebno pogost je pri domačih psih in mačkah, medtem ko ga pri parkljarjih in kopitarjih skoraj ni. To je kompleksna bolezen,

pri kateri sodelujejo genetski in okoljski dejavniki. Med 10 in 25-odstotki raka pri ženskah je dedno pogojenega, drugi primeri so sporadični. Poleg etnične pripadnosti je najbolj očiten faktor, ki vpliva na tveganje za razvoj raka mlečne žleze, nosečnost. Tveganje se pri ženskah, ki niso imele nobenega otroka, v primerjavi s tistimi, ki so imele prvega pred dvajsetim letom starosti zviša kar za polovico. Vsi ostali dejavniki, ki povečajo ali zmanjšajo tveganje za nastanek raka (izpostavljenost mutagenom, radiacija, prehrana, itd.) imajo največji učinek v času pubertete (Szpirer in Szpirer 2007).

Histološko je rak izjemno heterogen, najbolj invazivna oblika pri ljudeh metastazira v kosti, jetra, pljuča in mehko tkivo. Celične in biokemijske karakteristike, ki so pri raku mlečne žleze pomembne in so močno povezane s prognozo, so prisotnost receptorjev za estrogen in progesteron ($ER^{+/-}$, $PR^{+/-}$), bazalni ali luminalni fenotip, ter profil izražanja genov. Najslabša prognoza je pri raku z bazalnim fenotipom, ki nima receptorjev za steroidne hormone. Antiestrogenska terapija pri tej vrsti raka ni učinkovita. To je manj pogosta oblika raka, ki se pojavi pri 15 do 21 odstotkih žensk (Kumar in sod. 2007 in Stingl in Caldas, 2007).

Glavna in edina očitna skupna značilnost raka mlečne žleze pri ljudeh in pri različnih živalskih vrstah je njegova heterogenost. Prav ta heterogenost je številne znanstvenike, ki iščejo vzrok za nastanek raka mlečne žleze, pripeljala do hipoteze rakave matične celice. Dokazi v prid tej hipotezi se v zadnjem času intenzivno kopičijo. Ena izmed značilnosti raka mlečne žleze je tudi njegova ponovljivost. Rak se v visokem odstotku več let po končanem zdravljenju s kemoterapijo ponovno razvije. Nekatere od rakavih celic, ki so bolj odporne proti sevanju in kemoterapevtikom, preživijo in se kasneje ponovno razmnožijo (Dontu in sod., 2005). Eden izmed načinov klasifikacije raka je tudi profil izražanja genov z mikromrežami. Na ta način so identificirali 5 tipov raka pri ženskah, ki se značilno razlikujejo tudi po prognozi. Mikromreže zato postajajo splošna praksa pri diagnostiki raka na dojki.

4 RAKAVA MATIČNA CELICA

Po vsesplošno sprejetem stohastičnem konceptu raka kompaktnega tkiva velja, da so rakave celice heterogene, večina teh celic se lahko deli in tvori rakavo tkivo. Po teoriji rakave matične celice pa so obsežne proliferacije in tvorbe novega rakavega tkiva sposobne le določene celice – rakave matične celice, ki imajo vse lastnosti normalnih matičnih celic, razen sposobnosti natančnega uravnavanja celičnih delitev. Zanesljiv dokaz vprid tej teoriji je dejstvo, da je le majhna količina celic iz tumorja

sposobnih tvoriti nove tumorje po presaditvi v NOD/SCID miši. Glavno vprašanje pri nastanku raka mlečne žleze je, kdaj in kje pride do usodne mutacije, oziroma katere celice so primarne tarče maligne transformacije. Teorija rakave matične celice trdi, da do začetne usodne mutacije pride pri matični celici ali njihovih zgodnjih potomkah. Argumenti v prid tej teoriji so številni; 1) matične celice so dolgožive in so v tkivu dovolj dolgo, da akumulirajo mutacije, ki lahko vodijo do malignih sprememb, 2) normalne in tumorske celice imajo mnogo skupnih fenotipskih lastnosti, kot so nediferencirano stanje, zmožnost samoobnavljanja (asimetrična delitev), večja aktivnost zaščitnih mehanizmov (transporterji za izčrpavanje škodljivih snovi) ter zmožnost migracije, 3) dejavniki, ki povečajo ali zmanjšajo tveganje najmočnejše delujejo v času pubertete, ko so matične celice najbolj aktivne, 4) pri rakavih celicah so zelo pogosto našli okvare v genih signalnih poti, ki uravnavajo asimetrično delitev in nediferencirano stanje. Prav te signalne poti so odgovorne za pravilno delovanje matičnih celic in homeostazo tkiva, njihova vloga bo opisana v nadaljevanju (Ponti in sod., 2006).

Rakave matične celice imajo vse značilnosti normalnih matičnih celic, le da so te značilnosti pri rakavi celici posledica mutacij in so zato škodljive. Neposredna povezava med normalno in rakavo matično celico še ne obstaja, saj je slednja lahko posledica mutacije same matične celice, njenih multipotentnih potomk, ali celo diferenciranih celic, ki z mutacijo pridobijo lastnosti rakavih matičnih celic. Nekateri raziskovalci zagovarjajo možnost, da so primarne tarče transformacije prehodno deleže se multipotentne potomke matičnih celic, tako imenovane prednice. To je namreč populacija celic, ki se najbolj intenzivno deli, medtem ko so matične celice manj dinamične. Možno je tudi, da matične celice nosijo genetske napake, ki se fenotipsko izrazijo le pri celicah potomkah, ki se nato obnašajo kot rakave matične celice. Ker ima rakava matična celica nekatere markerje, ki so značilni za bazalne celice mlečne žleze, od koder izhajajo normalne matične celice, je kljub temu najverjetneje, da do maligne mutacije pride v matični celici ali eni od prednic. Ta nabor možnosti se lepo ujema s heterogenostjo rakavih sprememb v mlečni žlezi (Ponti in sod., 2006; Stingl in sod., 2006).

Prvi je rakavo matično celico skušal izolirati All-Hajj s sodelavci (2003), ki je z uporabo površinskih markerjev $EpCam^{+}CD44^{+}CD24^{-/nizko}$ izoliral populacijo celic, ki je po presaditvi v NOD/SCID miš tvorila rakave tvorbe. Le nekaj sto teh celic je bilo potrebnih za nastanek rakavih tvorb, medtem ko vse druge celice niti v velikih količinah niso tvorile tumorjev. Tumorje je tvorilo manj kot 1-odstotek celic v populaciji $EpCam^{+}CD44^{+}CD24^{-/nizko}$, kar pomeni, da matične rakave celice tvorijo

le izjemno majhen odstotek celotne mase rakavega tkiva. Brez markerja, ki bi bil značilen le za rakavo matično celico, je natančno količino teh v tkivu težko določiti, saj je možen vzrok za tako majhno ugotovljeno količino tudi neuspešna rast človeških celic po presaditvi v miš (Kelly in sod., 2007).

5 VLOGA NIŠE PRI NASTANKU RAKA

Pri raziskavah je zelo pomembno upoštevati dejstvo, da so v tkivu vse lastnosti matičnih celic in njihovih potomk močno odvisne od dejavnikov mikrookolja, ki tvorijo njihovo nišo. Celice, ki tvorijo nišo, imajo ključno vlogo pri pozicioniranju, proliferaciji in diferenciaciji matičnih celic, interakcije med različnimi celičnimi tipi pa so odločilne za pravilno homeostazo in funkcionalnost tkiv. Nekateri raziskovalci poudarjajo nišo matične celice kot najpomembnejši dejavnik pri nastanku raka mlečne žleze (Briskin&Duss 2007, in LaBarge, 2007). Dokazano je bilo, da potencialno tumorigene celice z resnimi genetskimi poškodbami lahko dolgo časa obstajajo v ugodnem mikrookolju, ko pa se to spremeni, se izrazi njihov tumorigeni potencial. Lep primer za to je študija pri ženskah, ki so bile v mladosti izpostavljene škodljivemu sevanju, v starosti pa so zbolele za rakom na dojki veliko pogostejše kot kontrolna skupina. Dokazano je bilo, da so nastanek raka sprožile spremembe v stromalnih celicah, ki tvorijo nišo. Vzrok za raka je lahko tudi lokalna sprememba v homeostazi tkiva zaradi kroničnega vnetja (Maffini in sod., 2004). Zelo prepričljiv dokaz za pomembnost niše v mlečni žlezi je bil poskus, v katerem so genetsko označene celice semenskih veziklov samčka miši skupaj z epiteljskimi celicami mlečne žleze presadili na očiščeno prsno maščobno blazinico. Celice semenskih veziklov so se v ustreznem mikrookolju reprogramirale in prispevale k vsem strukturam mlečne žleze (Boulanger in sod., 2007). Ko je celica vzeta iz konteksta svoje naravne niše, utegne spremeniti nekatere fenotipske značilnosti, vključno z izražanjem površinskih proteinov, kar lahko pogosto vodi v napačno interpretacijo rezultatov.

Pomembno je dejstvo, da matične celice nimajo receptorjev za steroidne hormone, prav ti pa regulirajo razvoj mlečne žleze. Edina možna razlaga tega fenomena je kompleksna interakcija matičnih celic z okoljskimi stromalnimi celicami v niši. Slednje zaznavajo hormonalne signale, ki jih s pomočjo topnih faktorjev na parakrini način posredujejo matičnim celicam. Za številne od teh faktorjev (npr: Wnt, Notch, TGF- β ...) je bila vloga pri nastanku raka mlečne žleze že dokazana.

6 ZAŠČITNA VLOGA NOSEČNOSTI

Že dolgo je znano povečano tveganje za nastanek raka pri ženskah, ki niso imele otrok, oziroma tistih, ki so imele prvega otroka po tridesetem letu starosti. Nosečnost torej močno zniža verjetnost za razvoj raka na dojki. Po nekaterih podatkih je ta po vsakem rojstvu za 11% manjša. Enako je pri miših, kjer opazimo zaščitni učinek tudi, če je brejost vmes prekinjena. Daljša je brejost, večji je njen zaščitni vpliv. Zaščitni učinek nosečnosti je povezan zgolj s primeri raka, ki imajo izražene estrogenske receptorje (ER⁺/PR⁺). Takih primerov je okrog 70-odstotkov in so povezani z boljšo prognozo, saj se za zdravljenje lahko uporablja antiestrogenska terapija kot npr, tamoxifen in inhibitorji aromataze (Britt in sod., 2007).

Navedeni podatki kažejo na pomembno vlogo hormonov pri nastanku raka. Na to kaže tudi statistika pojavnosti te oblike raka pri različnih živalskih vrstah. Najboljši primer so psi in mačke, pri katerih je, podobno kot pri ljudeh, večina tumorjev induciranih spontano, za razliko od miši kjer jih največkrat inducira virus. Pogostost te oblike raka je pri psih in mačkah še večja kot pri ljudeh, odstotek teh primerov pa se pri samicah z odstranjenimi jajčniki močno zmanjša. Na Norveškem, kjer pasjih samic skoraj nikoli ne sterilizirajo, do tumorskih sprememb pride pri več kot polovici samic. Verjetnost za pojav raka mlečne žleze je pri nesteriliziranih mačkah kar 7-krat večja kot pri steriliziranih. Po drugi strani pa je rak mlečne žleze pri divjih sorodnikih psov in mačk redek pojav (čeprav je treba dodati, da je pregled nad temi obolenji pri prosto živečih vrstah precej slabši). Enako velja tudi za vse rastlinojede živali, npr. domače kopitarje in parkljarje (govedo, koze, konji, ovce, prašiči), pri katerih se ta oblika raka skoraj ne pojavlja, ne glede na to ali gre za gojene ali prostoživeče živali. Različno pogostost med živalskimi vrstami lahko razložimo z različnimi reprodukcijskimi strategijami živali, ki pogojujejo količino in frekvenco vrhov steroidnih hormonov v krvi samic v teku življenja. Edini faktor, ki lahko pojasni razliko med divjimi živalmi in živalmi v ujetništvu ter se poleg tega ujema tudi s situacijo pri ženskah modernega sveta je število ovulacij, ki se ne končajo z gravidnostjo. Večina ovulacij pri divjih vrstah se namreč konča z brejostjo in laktacijo, domače nesterilizirane mačke in psi pa so vedno znova izpostavljeni ovarijskim steroidnim hormonom, ne da bi temu sledila laktacija. Osrednje vprašanje je, kateri hormon je za to odgovoren – estrogen, progesteron ali prolaktin. V preteklosti se je poudarjala vloga estrogena, danes pa podatki kažejo na večjo vlogo progesterona. Pri psih (imajo podaljšano lutealno fazo), se zdi bolj verjetna razlaga progesteron, pri mačkah (večinoma imajo inducirano ovulacijo) pa je verjetnejši vzrok za raka mlečne žleze estrogen. Pomemben je podatek, da

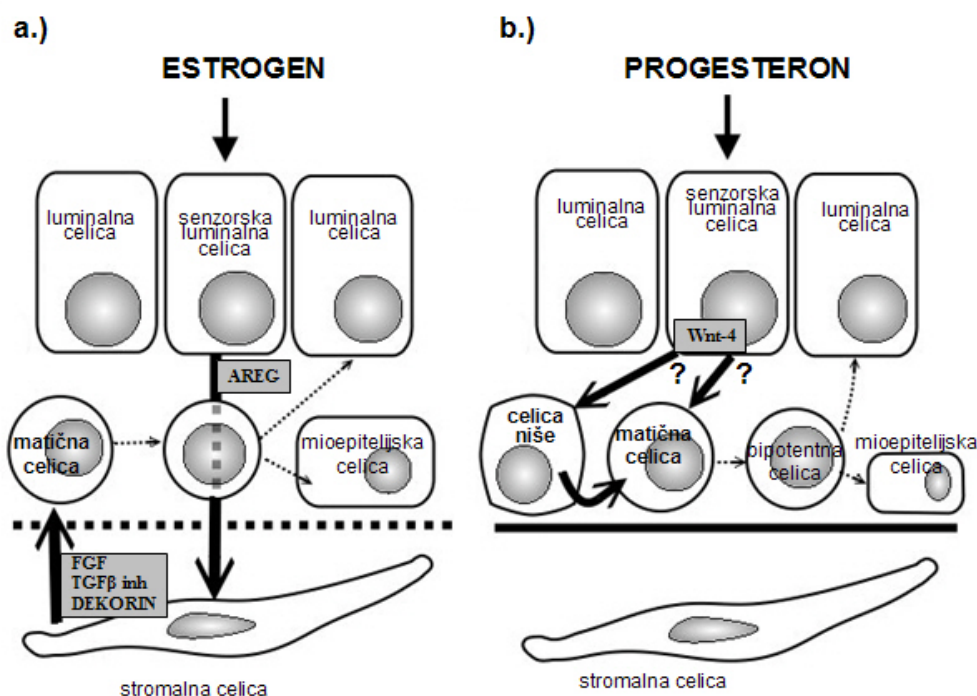
se je pri različnih vrstah velikih mačk v ujetništvu, ki so bile konstantno izpostavljene povišanim koncentracijam sintetičnega progesterona, rak mlečne žleze pojavil pri več kot 90-odstotkih živali (Munson in Moresco 2007).

Tretji hormon, ki je pomemben za ciklični razvoj mlečne žleze je prolaktin. Vir tega ni zgolj hipofiza, ampak nastaja tudi lokalno v tkivu mlečne žleze in sicer pod vplivom estrogena. Mnogo dokazov kaže na to, da avtokrino/parakrina prolaktinska zanka v tkivu mlečne žleze igra pomembno vlogo pri razvoju in napredovanju raka mlečne žleze. Sicer je povezava med rakom mlečne žleze in prolaktinom precej sporna, saj različni avtorji zastopajo dokaj različne trditve – nekateri trdijo, da je prolaktin sicer povezan s pojavnostjo benignih sprememb, pri malignih spremembah pa ni jasne povezave. Drugi avtorji celo omenjajo pozitivne učinke prolaktina (Goodman&Bercovich 2007; Duan in sod. 2007). Mehanizem zaščitnega vpliva nosečnosti na pojavnost raka

mlečne žleze ni znan, najbolj verjetno pa je posledica spremenjenega hormonskega stanja. Obstajajo modeli za razlage, ki temeljijo na kompleksni interakciji matičnih celic z okoljem niše.

7 MATIČNE CELICE, NJIHOVA NIŠA IN VLOGA HORMONOV PRI NASTANKU RAKA

Steroidna hormona estrogen in progesteron imata ključno vlogo pri razvoju mlečne žleze v puberteti in nosečnosti. Delujeta preko ustreznih receptorjev na luminalnih epiteljskih celicah. Njuna vezava sproži izražanje lokalnih topnih faktorjev, ki omogočijo vzpostavitev in delovanje niše. Različni poskusi so pokazali, da je za normalni razvoj mlečne žleze pod vplivom estrogena pomembna interakcija med senzorskimi celicami z



Slika 1: Hormonska regulacija matičnih celic v terminalnem brstu mlečne žleze. a.) V času pubertete se estrogen veže na receptorje senzorskih luminalnih celic, kar sproži izločanje amfiredulina. Ta deluje na stromalne celice pod bazalno membrano, ki je prekinjena. Stromalne celice izločajo faktorje, ki stimulirajo delitev matičnih celic. b.) Pri odrasli mlečni žlezi senzorske celice stimulira ovarijski progesteron, ki se izloča med estričnim ciklom ali gravidnostjo. Bazalna lamina je tu nepropustna, zato glavno vlogo v niši igrajo spremenjene mioepitelijske celice. Stimulirane senzorske celice izločajo faktor Wnt-4, ki stimulira matične celice k delitvi bodisi direktno ali pa preko celic niše.

Figure 1: Hormonal regulation of stem cells in the terminal bud of the mammary gland. a.) During the puberty estrogen binds to receptors of the luminal sensoric cells and causes secretion of amphiregulin, which acts on stroma cells under the ruptured basal membrane. Stroma cells excrete factors stimulating cell divisions of stem cells. b.) In the adult mammary gland the sensory cells are stimulated by progesterone, which is secreted during the oestric cycle or during pregnancy. The basal lamina is tight therefore the main role in the niche play modified myoepithelial cells. Stimulated sensoric cells excrete the Wnt-4 factor, which stimulates cell division of the stem cells either directly or through the cells in the niche.

estrogenskim receptorjem (ER⁺) in matičnimi celicami, ki tega receptorja nimajo. Glavno vlogo pri komunikaciji igra epidermalni rastni faktor amfiregulin, ter celice z receptorjem za amfiregulin (ang.: »EGFR-epithelial growth factor receptor«).

Glavna aktivnost med podaljševanjem mlečnih kanalčkov poteka v terminalnih brstih, kjer je v nezreli žlezi največja koncentracija matičnih celic. V času intenzivnega podaljševanja kanalčkov je bazalna membrana prekinjena, kar omogoča komunikacijo med epitelijskimi celicami in stromalnimi celicami vezivnega tkiva pod bazalno membrano. Glede na številne podatke o sintezi proteinov ter *in vitro* poskusih je najverjetnejši nasleni model interakcije celic v niši: estrogen se veže na luminalne senzorske celice, ki v odgovor izločajo amfiregulin, ta se veže na ustrezne receptorje stromalnih celic, stromalne celice pa so tiste, ki s parakrino stimulacijo sprožijo proliferacijo nad njimi ležečih matičnih celic (direktno ali indirektno). Najverjetneje pri tem sodelujejo nekatere metaloproteinaze in fibroblastni rastni faktorji (FGF2 in FGF7) (slika 1a). FGF so hkrati tudi ključna komponenta medija za gojenje kulture mamosfer. Seveda je celoten mehanizem mnogo bolj kompleksen in še ni do konca razjasnjen.

Na koncu pubertete, ko je mlečna žleza do konca razvita, glavno vlogo pri razvoju in morfogenezi le-te prevzame progesteron. Koncentracija progesterona se poveča med estričnim ciklom in nosečnostjo. Žlezni kanalčki v tem stanju dosežejo konec maščobnega tkiva in ne rastejo več, zato je bazalna lamina neprekinjena. Ker ni komunikacije med spodaj ležečimi stromalnimi celicami, njihovo vlogo verjetno prevzamejo mišično-epitelne celice. Luminalne celice ob vezavi progesterona izločajo topni faktor Wnt-4 in RANKL. Signalizacija z Wnt je zelo pomembna komponenta, ki vpliva na razvoj matičnih celic, RANKL pa preko ciklina D sproži razmnoževanje sosednjih luminalnih celic (slika 1b). Niši delovanja obeh hormonov se prekrivata – delita si iste hormonsko odzivne senzorske in matične celice, medtem ko so ostale celice niše različne (receptorje za steroidne hormone izraža več kot 30-odstotkov luminalnih celic žleze).

Delovanje estrogena preko EGFR in progesterona preko Wnt je podprto s številnimi dokazi. Kljub temu sta zgoraj opisana mehanizma le približen model, ki trenutno razlaga dogajanje med razvojem mlečne žleze, kot tudi povezavo med hormoni, topnimi parakrinimi faktorji (Wnt, Notch) in rakom mlečne žleze. Po številnih podatkih sta povišana izpostavljenost progesteronu zaradi hormonskih terapij, kot tudi povišano izražanje komponent Wnt signalizacije povezana z večjim tveganjem za nastanek raka mlečne žleze. Ob vsakem menstrualnem ciklu se s progesteronom preko Wnt stimulira delitev matičnih celic in nastanek večje količine prehodno de-

lečih se multipotentnih prednic, ki imajo velik proliferacijski potencial in so zato najbolj dovzetne za mutacije. Če temu ne sledi nosečnost in končna diferenciacija, se mutacije kopičijo, pri čemer se poveča možnost za maligno transformacijo, še posebej če imajo udeležene celice že akumulirane mutacije. To je ena izmed možnih razlag zaščitne vloge nosečnosti pred tveganjem za raka dojke (Briskens&Duss 2007; Wagner& Smith 2005).

8 OKVARA KONTROLNIH MEHANIZMOV MATIČNIH CELIC IN RAK

Pri tumorskih tkivih so zelo pogosto prisotne napake v regulaciji mehanizmov, ki sodelujejo pri vzdrževanju tipičnih lastnosti (samobnavljanje, nediferenciaro stanje) matičnih celic v različnih tkivih. Najbolj preučene so Wnt, Notch, Hedgehog, HER-2 in PTEN poti signalizacije. Kot že omenjeno, je Wnt pomemben posrednik pri aktivaciji matičnih celic v odzivu na progesteron, uravnava pa tudi proliferacijo prehodno pomnožujočih se prednic med razvojem mlečne žleze. Pri človeških celičnih linijah mlečne žleze, ki so bile transformirane za povišano izražanje Wnt-1 pride do maligne transformacije. Konstitutivna aktivacija Wnt rezultira v nastanku raka zaradi napačnega uravnavanja delitve matičnih celic in prednic. Močna aktivacija Wnt poti je povezana z mnogimi rakavimi spremembami, čeprav je mehanizem aktivacije lahko različen. To je lahko točkasta mutacija v genih za »downstream« regulatorje, (kot sta β -katenin in LRP5/6), ali pa povišana ekspresija Wnt, povzročena z onkogenim MMTV virusom (mouse mammary tumor virus), ki je pogosta pri miškah (Lindvall in sod., 2007).

V poskusih so opazili, da imajo transformirane celice s povišano ekspresijo Wnt tudi povišano aktivnost Notch signalizacije. Notch receptorska signalna pot omogoča medcelično komunikacijo, sodeluje pri apoptozi, določitvi usode celic in migraciji, ter omogoča asimetrično delitev matičnih celic. Povišana ekspresija Notch *in vitro* inhibira diferenciacijo normalnih celic epitelijske mlečne žleze, *in vivo* pa onemogoči razvoj mlečne žleze, ter povzroči nastanek rakavih tvorbo. Napačna Notch regulacija onemogoči terminalno diferenciacijo epitelijskih celic mlečne žleze, ter pospeši njihovo proliferacijo, kar povzroči motnje v homeostazi tkiva (Farnie&Clarke 2007).

Notch signalizacija je tudi posrednik avtokrine regulacije rakavih matičnih celic z IL-6. IL-6 je multifunkcijski citokin, ki se sprošča pri vnetjih in uravnava proliferacijo, preživetje in metabolizem celic. Sprva so znanstveniki opazili povezavo med slabo prognozo raka na dojki in povišano koncentracijo IL-6 v krvi, kasneje pa so opazili še povišano ekspresijo IL-6 v tumorskem

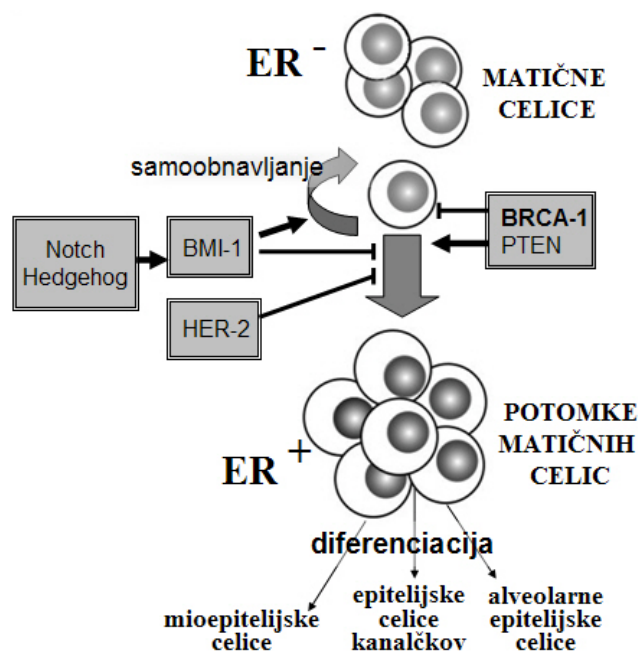
tkivu. Sansone s sodelavci (2007) je predlagal IL-6 kot potencialni regulator samoobnavitve pri normalnih in rakavih matičnih celicah. Maligna sprememba matične celice v mlečni žlezi sproži povišano izločanje IL-6, ki z avtokrinim delovanjem poviša izražanje liganda Jagged3. Ta se veže na Notch-3 receptor, kar sproži izražanje CA-IX, ki je karbonska anhidraza, ki naj bi omogočala celici, da preživi v hipoksičnem okolju rakavega tkiva. Avtokrina pozitivna povratna zanka tako pospeši in favorizira rast maligno spremenjenih celic (Schafer&Brugge, 2007).

Tudi Hengehog (Hh) je ligand, ki ga povezujejo z nastankom raka mlečne žleze. Visoka ekspresija Hh je značilna pri matičnih celicah, diferencirane celice pa ga skoraj ne izražajo. Hh preko regulacije »polycomb« gena BM-1 omogoča proces samoobnavljanja normalnih in rakavih matičnih celic v mlečni žlezi. Geni, ki so vključeni v Hh signalizacijo sami ponavadi niso mutirani, temveč pride do okvar v njihovih regulacijskih poteh in tako do povišanega izražanja. Stimulacija Hh poti v mlečni žlezi sproži nastanek hiperplastičnih lezij, za nastanek rakavih sprememb pa so največkrat potrebne še druge mutacije. Hh ligand tudi pospeši napredovanje rakavih sprememb in njihov razsev. Inhibitor Hh, ciklopamin vse te učinke izniči. Povišana aktivacija Hh signalne poti je povezana tudi z veliko ponovljivostjo raka na dojki po kemoterapiji. Njena inhibicija bi verjetno lahko zmanjšala ali preprečila ponovljivost raka na dojki (Hatsell&Frost 2007).

Eden zgodnejših molekularnih vzrokov za razvoj sporadične oblike raka je pomnoževanje ali povišana

ekspresija gena HER-2, iz družine epidermalnih rastnih faktorjev. Raziskave kažejo pozitivno korelacijo med količino matičnih celic in izražanjem HER-2. Slednji ima verjetno pomembno vlogo pri regulaciji populacije matičnih celic. Vse zgoraj našete signalne poti so trenutno glavna tarča pri iskanju zdravil za raka na dojki.

V kontekstu rakavih sprememb mlečne žleze je potrebno omeniti tudi pojem genomske stabilnosti, ki omogoča pravilno replikacijo DNA med celično delitvijo. Če je genom nestabilen, pride do prenosa spontanen napak v replikaciji ali z zunanjimi faktorji sproženih mutacij v naslednjo generacijo in posledično do rakavih sprememb. To je še posebej pomembno pri matičnih celicah, ki so sposobne regenerirati celotno tkivo. Zato imajo te poseben zaščitni mehanizem pred poškodbami DNA. Ob poškobi se namreč namesto popravilnih mehanizmov preferenčno sproži apoptoza, ali pa prehod celice v mirujočo fazo. Taka matična celica se sicer ne deli, vendar pa ostane v tkivu in se stara. Pridobi lahko drugačno funkcijo in tako spremeni mikrookolje v tkivu, ter povzroči maligno transformacijo drugih celic. Zato je vloga odziva na poškodbo DNA pri nastanku raka velika. Skoraj vsi do sedaj znani proteini, ki so povezani z genetsko predispozicijo za nastanek raka na dojki (BRCA1, BRCA2, PTEN, CHEK2...) imajo vlogo zaznavanja poškodb DNA v celici. Če poškodbe DNA matična celica ne zazna, to vodi k proliferaciji mutiranih celic in dodatni akumulaciji mutacij (Bartkova in sod., 2005). PTEN je lipidna fosfataza, ki je ključna za samoobnavljanje matičnih celic krvi in



Slika 2: Samoobnavljanje in diferenciacija pri matičnih celicah mlečne žleze. Dedna in sporadična oblika raka mlečne žleze izvirata iz matične celice ali njene zgodnje potomke. Začetni dogodek je napaka v regulaciji sicer natančno kontroliranega procesa samo-obnavljanja matičnih celic. Pri dedni obliki je najpogostejši vzrok izguba heterozigotnosti lokusa BRCA1, pri sporadični obliki pa mikrolelecija znotraj lokusa PTEN ali povišana aktivacija epidermalnega rastnega faktorja HER-2, ter Notch in Hedgehog signalne poti. Posledica tega je povečanje klonalnega razraščanja matičnih celic, kar pomeni nove tarče za nadaljne mutagene dogodke.

Figure 2: Self-renewal and differentiation of the mammary stem cells. Hereditary or spontaneous cancer in the mammary gland originates from stem cells or from their early progenitor cells. The initial event is a mistake in the regulation of precisely controlled process of self-renewal of the stem cells. For the hereditary form of cancer, the most common cause is the loss of heterozygosity at the BRCA1 locus and for the spontaneous form a microdeletion within the PTEN locus or elevated activation of the epidermal growth factor HER-2 and Notch and Hedgehog signal pathways. The consequence is increase of the clonal outgrowth of stem cells, resulting in new targets for further mutation events.

živčnega sistema. Mikrodelecija tega gena je prisotna kar pri polovici primerov raka na dojki in je največkrat posledica genomske nestabilnosti zaradi podedovane mutacije v *BRCA1*. Liu in sodelavci (2008) so v svojih študijah dokazali, da izguba heterozigotosti na lokusu *BRCA1*, ki je najpogostejši vzrok za dedno obliko raka na dojki, vodi v kopičenje genetsko nestabilnih matičnih celic. Te so podvržene dodatnim mutacijskim dogodkom, ki vodijo v razvoj raka.

Z razlago mehanizmov samoobnavljanja matičnih celic mlečne žleze se pokaže tudi konceptualna povezava med dedno in sporadično obliko karcinogeneze (slika 2).

9 RAZPRAVA

Zaradi pogostosti raka na dojki in njegove kompleksnosti se mnoge skupine znanstvenikov po svetu ukvarjajo z najrazličnejšimi vprašanji, povezanimi s to obliko raka. Internetna študija, v kateri so skušali postaviti prioritete pri raziskavah raka na dojki, je pokazala, da je iz sklopa 70 vprašanj, vprašanje vloge matičnih celic pri raku mlečne žleze na četrtem mestu. Zanimivo je, da neklinični raziskovalci to vprašanje smatrajo kot prioriteto (<http://breast-cancer-research.com/content/9/6/R81>). Na voljo je velika količina podatkov, vendar pa je včasih njihova interpretacija težavna, kar zmanjša tudi njihovo uporabno vrednost za klinične raziskave. To velja predvsem za podatke, pridobljene iz ekspresijskih analiz z mikromrežami, ki se uporabljajo tudi v diagnostične namene. Največja težava, ki predstavlja oviro pri tovrstnih raziskavah, je identifikacija ustreznih markerjev, s katerimi bi bilo mogoče iz mlečne žleze izolirati čisto kulturo matičnih celic. Trenutne metode namreč omogočajo le 5 % obogatitev kultur matičnih celic. Poznavanje specifičnih markerjev v hierarhiji celic mlečne žleze (od matičnih do diferenciranih celic) bi omogočilo učinkovite raziskave po vzoru raziskav vzrokov za nastanek raka krvnega sistema. Možno bi bilo ugotoviti natančno hierarhijo celic in linijsko diferenciacijo. Celice na različnih stopnjah diferenciacije bi lahko izolirali s tekočinsko citometrijo, določili njihov ekspresijski profil, jih *in vitro* genetsko spremenili ter nato ugotovili vpliv teh sprememb na delovanje v *in vitro* ter *in vivo* poskusih. Ekspresijski profil posameznih celic v hierarhiji bi omogočil tudi identifikacijo za določeno stopnjo specifičnih promotorjev in njihovo uporabo v transgenezi. Trenutno najpogostejše uporabljena promotorja (WAP in MMTV) močno omejujeta spekter možnosti.

Spontane maligne spremembe mlečne žleze miši kot model za karcinogenezo pri človeku niso najboljše, saj je, za razliko od človeka, pri miši vzrok rakave spremembe večinoma posledica prisotnosti onkogenega MMTV pro-

virusa. Tako nastale tvorbe imajo z rakavimi tvorbami pri ljudeh malo skupnih značilnosti. Za študije pri miših se zato uporablja PyMT MMTV miš. Tu se »*polioma middle T onkoprotein*« izraža pod kontrolo MMTV hormonsko odvisnega promotorja, ki se aktivira z estrogenom. Tako nastale rakave tvorbe so človeškim blizu po morfologiji in tudi po vzorcu izražanja genov in so dober model za raziskave. Vsekakor pa bi bil najboljši model študij kompleksnih, verjetno večstopenjskih dogodkov pri nastanku raka in vzpostavitev umetnega mikrookolja v mlečni žlezi. Ksenotransplantacijski postopki gojenja človeških tkiv mlečne žleze v miši so že znani, vendar so potrebne izboljšave modela (Proia&Kuperwasser 2006). Končni cilj je seveda določitev celičnih tarč različnih onkogenih mutacij in razkritje mehanizma nastanka rakave matične celice. Poznavanje teh mehanizmov bi olajšalo iskanje učinkovitejših zdravil in strategij za zdravljenje raka na dojki.

10 VIRI

- All-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J., Clarke M.F. 2003. Prospective identification of tumorigenic breast cancer cells. *Proceedings of National Academy of Science USA*, 100: 3983–3988
- Bartkova J., Horejsi Z., Koed K., Kramer A., Tort F., Zieger K., Guldberg P., Sehested M., Nesland J.M., Lukas C., Ørntoft T., Lukas J., Bartek J. 2005. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature*, 464: 864–870
- Briskén C., Duss S. 2007. Stem cells and the stem cell niche in the breast: an integrated hormonal and developmental perspective. *Stem cell reviews*, 3: 147–156
- Britt K., Ashworth A., Smalley M. 2007. Pregnancy and the risk of breast cancer. *Endocrine-related cancer*, 14: 907–933
- Dontu G., Liu S., Wicha M.S. 2005. Stem cells in mammary development and carcinogenesis. *Stem cell reviews*, 1: 207–213.
- Duan R., Ginsburg E., Vonderhar B.K. 2007. Estrogen stimulates transcription from the human prolactin distal promoter through AP1 and estrogen responsive elements in T47D human breast cancer cell. *Molecular and cellular endocrinology*, 281: 9–18
- Farnie G., Clarke R.B. 2007. Mammary stem cells and breast cancer-role of notch signalling. *Stem cell reviews*, 3: 169–175
- Goodman G., Bercovich D. 2008. Prolactin does not cause breast cancer and may prevent it or be therapeutic in some conditions. *Medical hypotheses*, 70: 244–251
- Hatsell S., Frost A.R. 2007. Hedgehog signalling in mammary gland development and breast cancer. *Journal of mammary gland biology and neoplasia*, 12: 163–173
- Huang E.H., Heidt D.G., Chen-Wei L., Simeone D.M. 2007. Cancer stem cells: A new paradigm for understanding tumor progression and therapeutic resistance. *Surgery*, 141: 415–419

- Kelly P.N., Dakic A., Adams J.M., Nutt S.L., Strasser A. 2007. Tumor growth need to be driven by rare cancer stem cells. *Science*, 317: 337
- Kumar M.J.M., Ponvijay K.S., Nandhini R., Nagarajan R.S., Srinivas J.J.G., Nagarajn P., Venkatesan R., Kumar K., Singh S. 2007. A mouse model for luminal epithelial like ER positive subtype of human breast cancer. *BMC cancer*, 7: 180
- LaBarge M.A., Petersen O.W., Bissell M.J. 2007. Of microenvironments and Mammary stem cells. *Stem cell reviews*, 3: 137–146
- Lindwall C., Bu W., Williams B.O., Li Y. 2007. Wnt signalling, stem cells and the cellular origin of breast cancer. *Stem cell review*, 3: 157–168
- Liu S., Ginestier C., Charafe-Jauffret E. *et al.* 2008. BRCA1 regulates human mammary stem/progenitor cell fate. *Proceedings of National Academy of Science in USA*, 105: 1680–1685
- Maffini M.V., Soto A.M., Calabro J.M., Ucci A.A., Sonnenschein C. 2004. The stroma as a crucial target in mammary gland carcinogenesis. *Journal of cell science*, 117: 1287–1290
- Ponti D., Zaffaroni N., Capelli C., Daidone M.G. 2006. Breast cancer stem cells: an overview. *European Journal of Cancer*, 42: 1219–1224
- Proia D.A., Kuperwasser C. 2006. Reconstruction of human mammary tissues in a mouse model. *Nature protocols*, 1: 206–214
- Sansone P., Storci G., Tavolari S., Guarnieri T., Giovannini C., Taffurelli M., Ceccarelli C., Santini D., Paterini P., Marcu K.B., Chieco P., Bonafè M. 2007. IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *The Journal of clinical investigation*, 117: 3988–4002
- Schafer Z.T., Brugge J.S. 2007. IL- 6 involvement in epithelial cancers. *The journal of clinical investigation*, 117: 3660–3663
- Szspirer C., Szspirer J. 2007. Mammary cancer susceptibility: human genes and rodent models. *Mammary Genome*, 18: 817–831
- Stingl J., Caldas C. 2007. Molecular heterogeneity of breast carcinomas and the cancer stem cell hypothesis. *Nature reviews, cancer*, 7: 791–799
- Stingl J., Eirew P., Ricketson I., Shackleton M., Vaillant F., Choi D. 2006. Purification and unique properties of mammary epithelial stem cells. *Nature*, 439: 993–997
- Vaillant F., Asselin-Labat M.L., Shackleton M., Lindeman G.J., Visvader J.E. 2007. The emerging picture of the mouse mammary stem cell. *Stem cell reviews*, 3: 114–123
- Wagner K.U., Smith G.H. 2005. Pregnancy and stem cell behaviour. *Journal of mammary gland biology and neoplasia*, 1: 25–36
- Welm B.E., Tepera S.B., Venezia T., Graubert T.A., Rosen J.M., Goodel M.A. 2002. Sca-1 cells in the mouse mammary gland represent an enriched progenitor cell population. *Developmental biology*, 245: 42–56

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- Fraser A.F., Broom D.M. 1990. Farm animal behaviour and welfare. London, Bailliere Tindall: 437 str.
- Hvelplund T. 1989. Protein evaluation of treated straws. V: Evaluation of straws in ruminant feeding. Chenost M., Reiniger, A. (ur.). London, Elsevier Applied Science: 66–74
- Žgajnar J., Kermauner A., Kavčič S. 2007. Model za ocenjevanje prehranskih potreb prežvekovalcev in optimiranje krmnih obrokov. V: Slovensko kmetijstvo in podeželje v Evropi, ki se širi in spreminja. 4. konferenca DAES, Ljubljana, 8–9 sep. 2007. Kavčič S. (ur.). Domžale, Društvo agrarnih ekonomistov Slovenije: 279–288
- ISO 5534 / IDF 4. Cheese and processed cheese – Determination of the total solids content – Reference method. 2004: 1–7
- Frajman P., Dovč P. 2004. Milk production in the post-genomic era. *Acta agriculturae Slovenica*, 84, 2: 109–119.
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- Fraser A.F., Broom D.M. 1990. *Farm animal behaviour and welfare*. London, Bailliere Tindall: 437 p.
- Hvelplund T. 1989. Protein evaluation of treated straws. In: *Evaluation of straws in ruminant feeding*. Chenost M., Reiniger, A. (ed.). London, Elsevier Applied Science: 66–74
- Žgajnar J., Kermauner A., Kavčič S. 2007. Model za ocenjevanje prehranskih potreb prežvekovalcev in optimiranje krmnih obrokov. In: *Slovensko kmetijstvo in podeželje v Evropi, ki se širi in spreminja*. 4. konferenca DAES, Ljubljana, 8–9 Sep. 2007. Kavčič S. (ed.). Domžale, Društvo agrarnih ekonomistov Slovenije: 279–288
- ISO 5534 / IDF 4. Cheese and processed cheese – Determination of the total solids content – Reference method. 2004: 1–7
- Frajman P., Dovč P. 2004. Milk production in the post-genomic era. *Acta agriculturae Slovenica*, 84, 2: 109–119.
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