Archiv Tierzucht 54 (2011) 5, 535-545, ISSN 0003-9438
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Changes in biochemical and hematological parameters and metabolic hormones in Tsigai ewes blood in the first third of lactation

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Abstract

The aim of this investigation is to determine changes in concentrations of biochemical and haematological parameters, as well as metabolic hormones in the blood of Tsigai ewes in the first third of lactation. The study included 10 ewes Tsigai breed monitored during three periods of lactation: 20 days, 40 and 60 days of lactation. Ewes were fed feed mixture (300 g/day) and meadow hay *ad libitum*. A significant decrease of concentrations of Ca and Na was recorded in the blood of sheep at the 40th day of lactation and later an increase at the 60th day of lactation. The opposite trend was determined for concentrations of P-inorganic. Also it was determined a significant decrease in Fe content and an increase in the concentrations of glucose, triglycerides and total protein in the first third of lactation. In the blood of ewes at 40th day of lactation it was found a significant decrease of the activity of AST and LDH in contrast to ewes at 20th day of lactation. Concentrations of T_3 and T_4 hormones were slightly increasing in the first third of lactation, but the differences were not significant. The blood insulin concentrations were significant increased in the first third of lactation. Haematological blood parameters in lactating ewes did not differ significantly and was within the reference values. Determining the concentration of biochemical and haematological parameters and concentrations of blood thyroid hormones and insulin in the first third of lactation are imposed as a precaution in order to better monitoring of Tsigai ewes during lactation.

Keywords: Tsigai ewes, lactation, blood, parameters, metabolic hormones

Zusammenfassung

Veränderungen der biochemischen und hämatologischen Parameter sowie von Stoffwechselhormonen im Blut erstlaktierender Tsigai Mutterschafe

Ziel der Untersuchung ist es, Veränderungen der Konzentrationen biochemischer und hämatologischer Parameter sowie von Stoffwechselhormonen im Blut von Tsigai Mutterschafe während des ersten Drittels der Laktation zu bestimmen.

Dazu wurden zehn Tsigai Mutterschafe 20, 40 und 60 Tage nach der Laktation kontrolliert.

Die Tiere bekamen eine Futtermischung (300 g/Tag) und Heu *ad libitum*. Eine deutliche Abnahme der Konzentration von Ca und Na im Blut wurde nach 40 Tagen festgestellt, am 60. Tag der Laktation jedoch wieder eine Zunahme.

Ein Gegentrend wurde für anorganisches Phosphat beobachtet. Im ersten Drittel der Laktation wurde eine signifikant sinkende Eisenkonzentration und steigende Glukose-, Triglyzerid- und Gesamteiweißkonzentration festgestellt. Die Aktivität von AST und LDH nahm nach 40 Tagen im Vergleich zu 20 Tagen signifikant ab. Auch die Konzentrationen von T_3- und T_4-Hormonen erhöhten sich leicht, aber nicht signifikant im ersten Drittel der Laktation, während sich der Insulinspiegel signifikant erhöhte. Die hämatologischen Parameter unterschieden sich nicht signifikant und blieben innerhalb der Referenzwerte.

Die Beobachtung der biochemischen und hämatologischen Parameter sowie von Stoffwechselhormonen im Blut im ersten Drittel der Laktation werden zur besseren Überwachung der Tsigai Mutterschafe während der Stillzeit empfohlen.

Schlüsselwörter: Tsigai Schaf, Laktation, Blutwerte, Stoffwechselhormone

Introduction

Tsigai is a traditional sheep breed, widely distributed in different variations across regions of central, Eastern and Southern Europe (Kusza *et al.* 2010). The adult body weight of the ewes in Croatia lies between 60 and 80 kg. Fertility of Tsigai ewes are between 110 and 160 %. Tsigai ewes are milked in average 180 day milking period and milk yields varied between 50 and 150 L (Antunović 2008). This breed is also characterized by its longevity and birth easiness. In 2009, the number of animals registred in the herdbook was of 3 000, which belonged to 19 herds (breeders). Lactation is a very demanding period for the animal when they are increased nutritional needs. During this period, especially in the first third of lactation, it is difficult to satisfy the nutritional requirements of animals because of high milk production. Especially are increased need for energy and minerals for milk synthesis. Because of it can come to significant changes in the animals-ewes. Changes occur not only in producing properties, but also lead to the certain metabolic disorders that affect on the concentration of minerals, biochemical and haematological parameters in the blood of small ruminants (Das & Singh 2000, Sobiech *et al.* 2008). All these changes indicate that the animals during lactation, particularly in its initial phase in metabolic stress (Azab & Abdel-Maksoud 1999). There are three items that must be secured in order to avoid the disease in animals in lactation: timely adaptation of the rumen in high-energy foods in lactation, maintenance of calcium concentration in the reference values and maintain a strong immune system (Goff & Horst 1997). Piccione *et al.* (2009) showed that during lactation the mammary gland secretory cells utilize 80 % of the blood-circulating metabolites for milk synthesis, depending on infiltration spend of precursors milk compounds (i.e. glucose, free amino acids and fatty acids). Metabolic hormones such as insulin and thyroid hormones also play a role in lipid metabolism. Monitoring the concentration of biochemical and haematological parameters as well as concentrations of thyroid hormones and insulin in the blood of sheep gives us a clearer picture of their nutritional and health status before the changes are visible on the animal (Carcangiu *et al.* 2007, Todini 2007, Antunović *et al.*

2009). The aim of this investigation is to determine changes in these indicators in the blood of Tsigai ewes in the first third of lactation.

Material and methods

Animals, locations of investigation and diets

Biological investigations were carried out with 10 clinical health Tsigai ewes, free from internal and external parasites and kept on one farm during winter season. Selecting sheep was carried out according to the registers from the flock of 200 sheep Tsigai breed. The criterion for selection was the age of sheep, lactation, sheep origin, litter size, sex and birth body weight of lambs in the litter. The selected ewes were fertilized with the same ram Tsigai breed from mentioned herd. The ewes were a median age of 4 years with one male lamb in litter with the average birth weight 4.53 kg. This study was conducted during 2009 at the family farm Ursic (Croatia, located 35 km south-east of Osijek, (42.150°N; 52.647°E). This area is located within the Baranja region and on altitude approximately 91 m. The ewes were divided into three periods according to the stage of lactation: 20th days, 40th and 60th days of lactation. The ewes were fed meadow hay (*Lolium perenne, Lolium italicum, Phleum phleoides, Trifolium repens and Dactylis glomerata*) *ad libitum* and 300 g of feed mixture (corn: 55 %, wheat bran 15 %, molasses 4 %, soybean grits 9 %, sunflower greats 9 %, livestock yeast 2 %, limestone 2 %, premix 2.5 %, mono-calcium phosphate 1 % and salt 0.5 %, with 16.00 % crude proteins and 15 MJME/kg) during investigation. Water was provided *ad libitum* to all ewes for the entire trial.

Sampling and analyses

The mean body weights and standard deviation of lactating ewes during 20th, 40th and 60th days of lactation were 74.10±5.61 kg; 72.85±10.28 kg and 70.10±7.64 kg. The body condition scores (BCS) of ewes (1=emaciated to 5=obese) was evaluated by two trained technicians according Russel (1991). The mean BCS and standard deviation of lactating ewes during 20th, 40th and 60th days of lactation were 2.48±0.65, 2.35±0.35 and 2.25±0.38. Blood was collected from the jugular vein (10 ml) into serum Vacutainer tubes (Venoject, Sterile Terumo Europe, Leuven, Belgium) and ethylenediamine tetra-acetic acid (EDTA) after morning feeding. The EDTA tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant. Blood serum was separated by centrifugation (10 min) at speeds of 3 000/min^{-1}. Concentrations of electrolytes (calcium [Ca], phosphorus[P]-inorganic, potassium [K], sodium [Na], magnesium [Mg], iron [Fe] and chloride [Cl]), biochemical parameters (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, creatinine, total protein, albumin and total bilirubin) and enzyme activity (alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatine kinase [CK], γ-glutamyl transferase [GGT] and lactate dehydrogenase [LDH]) were determined with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland, manufactured for Olympus Diagnostic GmbH, Hamburg, Germany using OLYMPUS AU 600 apparatus. GSH-Px was determined in whole blood of sheep with commercial »Ransel« kit (Randox Laboratories Ltd, London, UK). Method for determination

of (glutathione peroxidase) GSH-Px is based on catalytic oxidation of glutathione by cumen hidroksi peroxide, and for reading is used spectrophotometer UV/VIS JENWAY 6305. Determination of hematological parameters (number of leukocytes [WBC] and erythrocytes [RBC], as well as content of hemoglobin, hematocrit, mean corpuscular volume [MCV], the average hemoglobin content in erythrocytes-MCH and mean hemoglobin concentration in erythrocytes [MCHC]) in whole blood of sheep was carried out on an automatic three diff hematology analyzer Sysmex PocH-100iV. A differential blood test was carried out by microscope using the prepared blood smears coloured by Pappenheim. Concentrations of total T_3 and T_4 in blood serum were determined by means of duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, USA) by Imx-Abbott immunoanalyser. Methods for determination of T_3 and T_4 were MEIA (Microparticle Enzyme Immunoassay) and FPAI (Fluorescence Polarization Immunoassay). Sensitivity of the assay was less than 0.4 nmol/L (T_3) and 12.8 nmol/L (T_4). Mean recovery rates were 98.6 %. Serum insulin concentrations were analyzed by MEIA on Abbott Axsym Systems (Abbott Laboratories, USA).

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture, Forestry and Water Management.

Statistical analysis

The results were statistically evaluated by using repeated measures test (Statistica version 8, StatSoft [2008]). Differences were considered as significant at the level of 0.05 or less. The means and theirs standard deviation (SD) as well as standard errors (SE) were also calculated. Pearson's correlation method was used to assess the relation between the different biochemical parameters and thyroid hormones for three stage of lactation.

Results and discussion

A significant decrease concentration of Ca and Na was recorded in the blood of ewes 40th days and later their growth during the 60th days of lactation. The opposite trend was found for concentrations of P-inorganic (Table 1). Fe concentrations in the blood of ewes were significantly decreased ($P<0.05$) in the first third of lactation, which may be associated with increased synthesis of milk and an increased need for Fe. The decrease in Ca concentration in the blood can cause feeding animals with grains of wheat, and when the animal is restricted or completely prevented from grazing (Larsen *et al.* 1986). However, Liesegang *et al.* (2007) stated that the cause of the fall of calcium concentration in the serum of ewes after birth and in early lactation may be associated with increased secretion of Ca through milk and its rearrangement in bone. To the similar conclusions have come and Carcangiu *et al.* (2007). Abdelrahman *et al.* (2002) they also found lower concentrations of Ca in the blood of ewes in lactation compared with the reference values. The increase P-inorganic concentrations in the serum of ewes after birth were found Ozyurtlu *et al.* (2007) and goat Tanritanir *et al.* (2009). Similar results for the concentration of Ca and P-inorganic during lactation in the

blood of crossbreed ewes (Istrian × East Friesian) were found Mašek *et al.* (2007). In addition Ca mobilize from bone and P through a similar path, but far more Ca from the body/blood excreted in milk, P also, but not so much, and consequently his concentration in the blood is higher. During the 40th days of lactation were found lower concentrations of Na, Mg and Cl in the blood of ewes. The reason for this may be increased secretion of electrolytes with milk which is to be expected given the high production of milk in this period of lactation. Similar results determined Baranowski (1994). Although Hu & Murphy (2004) noted that the concentration of chloride is most dependent on their nutrient intake. Maintaining a constant level of K is a prerequisite for homeostasis, since substantial fluctuation in the content of this electrolyte may lead to structural and functional disorders: e.g. cardiac muscle, smooth muscles, and skeletal muscle (Williams *et al.* 2004).

Table 1
Effect of stage of lactation on the concentration electrolytes in the blood of ewes

Parameter	Stage of lactation			SE
	Mean±SD			
	20 day	40 day	60 day	
Ca, mmol L^{-1}	2.59^{A}±0.07	2.42^{B}±0.08	2.69^{C}±0.07	0.02
P-inorganic, mmol L^{-1}	$1.61A^{a}$±0.52	2.18^{B}±0.18	1.91^{Ab}±0.16	0.04
K, mmol L^{-1}	5.83±0.40	5.55±0.51	5.67±0.46	0.08
Na, mmol L^{-1}	162.30^{A}±2.21	154.10^{B}±2.13	164.30^{AC}±3.20	0.94
Mg, mmol L^{-1}	0.91±0.16	0.83±0.17	0.97±0.19	0.06
Cl, mmol L^{-1}	115.80±0.92	111.20±1.81	117.00±1.49	0.53
Fe, µmol L^{-1}	22.70^{a}±3.95	19.27^{ab}±3.62	18.18^{b}±4.59	0.80

$^{a,b}P<0.05$, $^{A,B,C}P<0.01$, SD: standard deviation, SE: standard error

Table 2 shows the concentrations of biochemical parameters in blood of ewes at different stages of lactation.

Table 2
Effect of stage of lactation on the concentrations of biochemical parameters in blood of ewes

Parameter	Stage of lactation			SE
	Mean±SD			
	20 day	40 day	60 day	
Glucose, mmol L^{-1}	3.26^{a}±1.73	3.86^{ab}±0.49	4.30^{a}±0.64	0.10
Urea, mmol L^{-1}	6.80±0.46	6.94±0.35	7.43±0.59	0.22
Uric acid, µmol L^{-1}	18.00±6.46	20.00±7.10	21.20±6.98	1.49
Cholesterol, mmol L^{-1}	1.57±0.28	1.54±0.23	1.69±0.20	0.04
HDL-cholesterol, mmol L^{-1}	1.09±0.23	1.09±0.17	1.18±0.16	0.03
LDL-cholesterol, mmol L^{-1}	0.40±0.08	0.36±0.08	0.40±0.08	0.02
Triglycerides, mmol L^{-1}	0.19^{a}±0.06	0.20^{ab}±0.01	0.25b±0.09	0.01
Creatinine, µmol L^{-1}	79.20±11.71	76.50±7.04	76.60±8.06	1.63
Total proteins, g L^{-1}	72.50±4.50	75.45±4.11	76.11±4.80	0.88
Albumine, g L^{-1}	28.60±3.80	29.21±0.82	29.48±1.59	0.43
Total bilirubin, µmol L^{-1}	3.10±0.57	3.00±0.11	3.00±0.12	0.06

$^{a,b}P<0.05$, SD: standard deviation, SE: standard error

It was found that with increasing lactation significantly increased ($P<0.05$) concentrations of glucose and triglycerides as well as also found increased concentrations of urea, uric acid, cholesterol, total protein and albumin, but these differences were not significant ($P>0.05$). HDL and LDL-cholesterol, creatinine and total bilirubin in serum of ewes are not varied depending on the stage of lactation. A significant increase in concentration of glucose, total protein, triglyceride and urea, as stage of lactation advances in the blood of Kamieniec ewes determined Sobiech *et al.* (2008). Increasing concentrations of glucose may be associated with increased milk production due to the early lactation and increased activity of mammary gland increase the energy needs for almost four times (Block *et al.* 2001). It is well known that in ruminants during lactation needs of tissues for insulin are reduced, which can cause a temporary increase in the concentration of glucose in the blood serum and thus to stimulate milk production (Szczepański *et al.* 2005). Higher concentrations of total protein in the blood of ewes in the later stages of lactation were found Karapehlivan *et al.* (2007) and Antunović *et al.* (2002). The reason for this may be a decrease in the concentration of globulin (El-Sherif & Assad 2001). The increase in triglycerides during lactation in the blood of ewes was determined by Mašek *et al.* (2007). The same authors state that as the stage of lactation advanced leads to reduced milk production and, thus, reduced milk fat synthesis. The reason for the increase in concentration of triglycerides in the blood of ewes during lactation may be associated with negative energy balance that accompanies the increased mobilization of fat in adipose tissue (Sobiech *et al.* 2008).

Activities of enzymes in the blood of lactating ewes were within the reference values (Kaneko *et al.* 2008), except GGT activity, which was slightly higher (Table 3). This could be due to a more intense liver function of lactating ewes so as to meet the energy and protein requirements for maintenance and milk production (Roubies *et al.* 2006). A significant decrease in activity of AST and LDH were detected in the blood of ewes during the 40th days of lactation compared to 20th days of lactation. The decrease of AST activity in the blood of ewes as the stage of lactation advances determined Castillo *et al.* (2007) and Mašek *et al.* (2007). Highest AST activity was detected in the blood of ewes 20th days of lactation, when we expect the highest milk production. Enhanced activity of AST indicates the stimulation of hepatic functions associated with higher productivity. Patkowski *et al.* (2006) have observed lower ALT activity and similar AST activity in the blood of sheep during 40th day of lactation. The activity of CK and GSH-Px did not differ depending on the stage of lactation, although there was a moderate increase during the lactation. Similar changes in activity of CK in the blood of goats as lactation progressed determined Mbassa & Poulsen (1991). GSH-Px activity in blood of lactating ewes indicates an adequate supply of selenium.

Higher concentration of T_3 was observed in the blood of ewes 40th days of lactation than at the 20th day of lactation, but these differences were not significant ($P>0.05$). A smaller increase in concentration of T_4, but no significant differences depending on the stage of lactation, was found in the blood of ewes in the first third of lactation (Table 4). These changes of thyroid hormone indicate an energy imbalance of ewes in early lactation. Similar concentrations of T_3 and T_4 in blood of ewes during lactation determined Karapehlivan *et al.* (2007). Pezzi *et al.* (2003) determined lower concentrations of T_3 and T_4 in blood of ewes at the beginning of lactation and their increased during lactation. To the similar results in lactating ewes and cows came Bekeova *et al.* (1993) and Sinka *et al.* (2008). Thyroid hormone

stimulates oxygen consumption, protein metabolism and milk yield. An increase in the level of thyroid hormones might be considered as indicator for tissue protein catabolism (Goldberg *et al.* 1980). These changes indicate on energy imbalance ewes in lactation, particularly in early lactation. Connection between thyroid hormone concentrations and energy balance determined Todini *et al.* (2007). Determined concentration of insulin in the blood of ewes suggest stated. Specifically, the lowest concentration of insulin found in the blood of ewes in early lactation (20 days), that they would later significantly increased as stage of lactation advanced. To the similar results in studies with lactating cows came Accrosi *et al.* (2005) and Eryavuz *et al.* (2008).

Table 3
Effect of stage of lactation on the activity of enzymes in the blood of ewes

Enzyme, U L^{-1}	Stage of lactation			SE
	Mean±SD			
	20 day	40 day	60 day	
ALT	15.70±3.68	16.90±3.48	17.70±2.41	0.59
AST	114.20^{AC}±10.50	89.00^{B}±9.74	104.90^{C}±15.85	2.91
CK	83.00±22.52	86.70±18.17	89.90±12.45	9.86
GGT	83.50±65.27	62.10±8.01	65.30±6.55	6.95
LDH	469.20^{a}±81.69	407.20^{b}±43.64	440.20^{ab}±63.06	12.33
GSH-Px	57 508.64±17 584	60 918.44±17 345	63 768.43±14 560	3 215

$^{a, b}P<0.05$, $^{A,B,C}P<0.01$, SD: standard deviation, SE: standard error

Table 4
Effect stage of lactation on the concentrations of metabolic hormones in blood of ewes

Hormon	Stage of lactation			SE
	Mean±SD			
	20 day	40 day	60 day	
T_3, nmol L^{-1}	1.26±0.11	1.43±0.11	1.36±0.15	0.07
T_4, nmol L^{-1}	43.48±18.18	46.37±9.29	45.38±8.99	3.59
Ratio T_3:T_4	0.030±0.006	0.032±0.005	0.031±0.006	0.004
Insulin, pmol L^{-1}	12.82^{a}±5.35	14.71^{ab}±5.84	18.74^{b}±5.76	0.91

$^{a, b}P<0.05$, $^{A,B}P<0.01$, SD: standard deviation, SE: standard error

Determined significant correlation of most biochemical parameters in blood of ewes in lactation, particularly in early lactation (20 days) suggest an association between these indicators, particularly metabolites involved in the metabolism of fats, proteins and minerals (Table 5).

The analysis of hematological parameters in blood of ewes as stage of lactation advanced determined is slight decrease in the concentration of WBC and RBC and hemoglobin content (Table 6). The decline in leukocyte counts during lactation in goat blood indicated their migration from blood into milk for more efficient phagocytosis and mammary gland defence against pathogens (Paape *et al.* 1992). In studies with lactating goats was also found a smaller decrease of blood leukocytes with the progress of lactation, but differences were not significant (Das & Singh 2000). Similar content of leukocytes in lactating goats found Iriadam (2007). To the similar changes content of RBC in the blood of goats in early lactation

came Azab & Abdel-Maksoud (1999). Similar results for hemoglobin content in lactating desert ewes determined Abdelatif *et al.* (2009). As the lactation progressed, the number of lymphocytes has grown, and highest was on the 40th days of lactation. The monocytes and basophiles numbers were minimal in blood of ewes during at beginning of lactation. Similar findings in goat founded Das & Singh (2000). The changes of all hematological parameters in blood of ewes in the first third of lactation were very small and were in the physiological values for sheep.

Table 5
Significant correlations between investigated parameters in the blood of sheep at different stages of lactation

Ratio	20th day of lactation	Ratio	40th day of lactation	Ratio	60th day of lactation
K:LDL	0.676	CHOL:HDL	0.967	CHOL:Cl	0.736
CHOL:Fe	0.643	CHOL:LDL	0.773	CHOL :HDL	0.923
CHOL: HDL	0.980	TRG:Fe	0.749	CHOL:LDL	0.624
CHOL:LDL	0.792	TRG:TBIL	0.881	UREA:Cl	0.696
LDL: Fe	0.671	Na:KRE	0.758	UREA:TP	−0.759
LDL: K	0.676	Na:ALB	0.757	TRG:TBIL	−0.904
LDL:KRE	0.732	TP:Ca	0.698	TRG:ALB	0.903
LDL:HDL	0.692			TP:KRE	0.659
UREA:KRE	0.677				
TRG:Ca	0.735				
TRG:Cl	0.682				
TRG:TP	0.717				
Na:ALB	0.734				
TRG:T_4	0.664				

CHOL: cholesterol, LDL: LDL cholesterol, HDL: HDL cholseterol, KRE: kreatinine, TRG: tryglicerides, ALB: albumine, TBIL: total bilirubin

Table 6
Effect of lactation stage on hematological parameters in blood of ewes

	Stage of lactation Mean±SD			SE
	20 day	40 day	60 day	
Parameter				
WBC, $\times 10^9$ L^{-1}	10.49±2.32	9.48±2.73	8.95±2.34	0.45
RBC , $\times 10^{12}$ L^{-1}	10.11±1.33	10.44±1.32	9.87±1.41	0.24
Hemoglobin, g L^{-1}	111.40±11.97	112.10±12.07	108.70±12.89	2.19
Hematocrit	0.44±0.05	0.43±0.06	0.42±0.06	0.01
MCV, fL	43.55±2.01	41.66±2.63	42.09±2.10	0.43
MCH, pg	11.04±0.51	10.77±0.51	11.08±0.77	0.11
MCHC, g L^{-1}	253.90±9.68	258.90±14.15	263.30±16.88	2.55
Distribution of leukocytes, %				
Lymphocytes	60.60±8.50	70.88±8.78	67.40±11.82	1.94
Neutrophils	23.90±3.93	18.56±3.84	20.10±9.18	1.20
Eosinophils	15.20±6.63	10.22±6.09	12.40±6.96	1.24
Monocytes	0.28±0.04	0.38±0.06	0.42±0.09	0.06
Basophils	0.12±0.03	0.10±0.04	0.15±0.06	0.05

SD: standard deviation, SE: standard error

In conclusion, monitoring the concentration of biochemical and haematological parameters as well as concentrations of thyroid hormones and insulin in the blood of ewes in lactation, particularly in early lactation, when the ewes significantly increased need for energy due to increased synthesis of milk should be introduced as one of quality procedures with the aim of complete control over a very demanding physiological stages of life.

Acknowledgements

This Study forms part of a research project 079-1780469-0225 financed by the Ministry of Science, Education and Sport of the Republic of Croatia.

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Received 10 August 2010, accepted 7 June 2011.

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