Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: en trámite

Vol.1 (1): 20-26

Comparison of weight increase in *Cambarellus montezumae* (Saussure, 1857) fed with a diet enriched with probiotic.

Castro-Mejía J*, Castro-Mejía G, Alcántara-Muñiz NF, Pacheco-Gómez V and Rodríguez- Estrada E.

Universidad Autónoma Metropolitana, Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo. Calzada del Hueso 1100, Col. Villa Quietud, C. P. 04960. Tel. (55) 5483-7151

*camj7509@correo.xoc.uam.mx

SUMMARY

An experiment was undertaken with 70 crayfish (*Cambarellus moctezumae*) obtained at Xochimilco Channels (CIBAC), using an artificial diet enriched with a probiotic (Lactobacillus), and as control, the same diet but without probiotic. The experiment was performed for 24 weeks in two acrylic tubs (35org/tub) of 1 m^2 x 0.40 cm depth, with 200 L of water. The temperature was kept at 25º±2ºC. Each week the organisms were weighted and measured and at the completion of the experiment the relative growth Rate (TCR) and the condition Factor (KM) would be determined. The average weight of the crayfish at the beginning of the experiment was 0.560 ±0.160 g. At week 24 of the experiment, the organisms fed with the enriched diet reached a weight of 3.167 ±0.156 g and with the control diet 1.292 ±0.112 g. The TCR values of the diet with probiotic indicate that major growth occurs within the first 8 weeks, while in the case of the control diet, it can be observed at the completion of the experiment. This can be corroborated with the KM. The enriched diet allowed organisms to have a better wellbeing along the whole experiment, on the contrary, with the control diet, the benefit is slightly observed on week 24. The main objective of this investigation is the addition of probiotics to the diet of these organisms in order to obtain a major gain of weight in order to be able to solve the problems raised by the commercial exploitation thereof.

Key words: *Cambarellus montezumae,* TCR, KM, Probiotics.

INTRODUCTION

Within aquaculture activity, the growth and the fattening of organisms are phases that can be considered as overcome in most cultures. The ecological and economical importance of crustaceous has conducted for the last years to further deepen in the knowledge of their biology as a basic requirement for the efficient use thereof. Crayfish are one of the few crustaceous that live in brooks, continental lacustrine deposits. They live both in temperate as well as in tropical climate, therefore, they are the most important, big and long-lived members of macro benthonic sweet aquaculture communities, and for this reason, they have successfully invaded a great variety of habitats, are resistant to humidity and temperature variations and present important adaptations that allow them to subsist even if body waters dry up (Rodríguez 1991). This development has been based more in a practical knowledge such as shrimp and crayfish (Rodríguez and Carmona 2002).

Cambaroides crayfish, given its characteristics, is considered as a great potential organism for culture and it has been affirmed that these are the only crustaceans that have been successfully cultivated in temperate regions (Iheu and Bernardo 1993). Crayfish are invertebrates that dominate macro-benthos of different latitudes in the world. There are approximately 500 species divided into three families: Astacidae, Cambaridae and Parastacidae, among which the genera such as *Astacus*, *Austropotamobius*, *Cherax*, *Euastacus*, *Orconectes*, *Pacifastacus* and *Procambarus* have a commercial and recreational importance (Marshall and Orr 1960)

Crayfish has various nourishment uses as it is consumed in rural populations in different parts of

Eceived: June 1, 2011. Accepted: September 1, 2011. Published: November 1, 2011.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: en trámite

Vol.1 (1): 20-26

the world, specially in ethnic groups, in Europe, in the United States as a luxury dish as it is greatly accepted by high income populations. Investigations about nourishment requirements for crustaceans are relatively recent, and definite solutions that may solve the problems posed by commercial exploitation have not been reached yet (Marshall and Orr 1960).

One aspect that should be considered for the success of this culture is nutrition (Castro *et al*. 2003). It is frequently observed that the food employed does not have the nutrients that species require for an optimum growth, mainly in the first part of their life, which is critical in all species, as it is here where major mortality can occur. At a global level, inert food is used with well-balanced nutritional ingredients, but there is also the possibility to use living organisms, susceptible to be modified in their nutritional contents (Latournerié *et al*. 2006).

Therefore, the objective of this investigation was to increase the weight and size of wild crayfish using an artificial diet enriched with a probiotic based on lactobacillus, obtained from the digestive tract of the angel fish *Pterophyllum scalare*.

MATERIAL AND METHODS

70 wild crayfish were obtained from the species *Cambalellus montezumae* from Xochimilco channels adjacent to the Centro de Investigación Biológica y Acuícola de Cuemanco (CIBAC), which were placed in two 1.0 m diameter and 0.40m depth acrylic tubes, filled with 200L of water (35 organisms/tube). Temperature was kept at 25º ± 2ºC, the pH in 6-7 and the oxygen at a concentration over 2 mg/L. In order for the organisms to have protection liars, 20 sections of 2.0 cm diameter and 5cm long were installed, as well as the plant *Ceratophyllum demersum* (hornwort).

In order to feed crayfish, a control diet was prepared. The material used to elaborate the diet is described in table 1.

The ingredients shrimp, broccoli and carrot were boiled separately until the shrimp became Pink and the broccoli and carrot were tender (a fork penetrates without difficulty). At the same time 10g of bacteriological agar were dissolved in 500 mL of hot water. Once the agar was dissolved, the container was placed onto a heating surface to boil the solution three or four times until the medium was clear.

Table 1. Diet formulation of an experimental diet for crayfish based on shrimp and bacteriological agar.

Ingredients	Quantity (g)
Small Shrimp*	400
Broccoli*	200
Carrot*	100
Apple	100
Tabasco banana	100
Kiwi	100
Calcium	0.5
Vitamins and minerals	0.5
B complex	0.5
Bacteriological Agar	10 g

*Previously boiled

Once the bacteriological agar is prepared, in a 2L capacity Blender, 100mL of water were added and all humid ingredients were blended, then the dry components were added. Once the nutritionals components were blended, 500 mL of bacteriological agar were added, finally adding 100 mL of lactobacillus solution (La3), previously obtained from the digestive tract of the angel fish *Pterophyllum scalare* (Monroy *et al*. 2009). The material was placed in plastic coolers in order to obtain 28 ±1 g of food cubes.

The weight and size average of crayfish at the inception of the experiment was 0.560 ±0.160 g and 2.585 ±0.283 cm. The organisms were weighted and measured (final length from beginning of face to end of furca) every week, for 24 weeks. In the same week of weight and total length measurement, tubs were cleaned to maintain optimum culturing conditions.

With the data of the weight and the total length, average weights and sizes were obtained, relative growth rate (TCR), as well as the degree of wellbeing (KM) of the population, in each of the 24 weeks of the experiment. The formulas used were taken from the experiment performed by Castro *et* *al*. (2009) and the formula of the condition factor of Medina (1979).

Relative growth rate (RGR)

$$RGR = \frac{\text{(Final weigth or length) - (Initial weigth or length)}}{\text{(Initial weigth or length) x (Final time - Initial time)}} \times 100$$

Instant growth rate (IGR)

$$IGR = \frac{\text{(Log final weigth or length) - (Log initial weigth or length)}}{\text{(Final time) - (Initial time)}} \times 100$$

Condition factor (CF)

$$CF = \frac{\text{Weigth}}{\text{Length}^3} \times 10^5$$

With respect to the statistical analysis performed to the weight and size of the individuals in every week of the experiment, in order to ensure the normality of the information, an analysis of a stem and leave was performed, as well as Box Plot. In addition, a descriptive analysis was performed to obtain the average values and the ± D.S. thereof. A two-channel variance analysis (ANDEVA) was applied to the values every four weeks to determine the existence of significant differences ($p < 0.05$) (Kachigan 1991, Tatsuoka 1970). The SYSTAT 10 program (Systat Software Inc., Calif. USA) was used for the statistical handling of the information.

In addition, a regression analysis was performed (Sokal and Rohlf 1981) both for the rate of growth of the weight and the length, with the help of the statistical program Excel 2003 (Microsoft Corp. Washington, USA).

RESULTS

The average weight values every four weeks of experimentation are included in table 2.

The organisms of the control diet presented a gain in weight of only 0.762 g; while those fed with the enriched diet was 2.607 g (Fig. 1). With respect to the relative growth rate, the organisms with the control diet present a major weight increase towards the end of the experiment; while animals fed with a diet with probiotic, the increase is present along the weeks, the highest levels occur during the first eight weeks of the experimentation (Fig.2).

With relation to the condition factor or degree of wellbeing of the population, regarding the size and weight, these indicate that the control diet does not supply the necessary requirements in order for the size to correspond to the weight of the animals, but until the completion of the experiment (24 weeks); while the organisms fed with an enriched diet the values are over the initial KM, which are maintained along the whole experiment (Fig.3).

Regarding the survival of the organisms, the enriched diet presented a value of 90%, while the organisms fed with the not enriched diet reached only a 60%.

Table 2. Weight (g) and total length (cm) mean values (±S.D.), of *C. montezumae* along 24 weeks of experimentation.

Week	Size	Weight of crayfish	
		Control diet	Diet + probiotic
0	2.585	0.560	0.560
S.D.	±0.328	±0.283	±0.160
4	2.860	0.609	0.890
S.D.	±0.123	±0.132	±0.158
8	3.020	0.662	1.319
S.D.	±0.134	±0.143	±0.149
12	3.106	0.759	1.781
S.D.	±0.162	±0.126	±0.153
16	3.168	0.995	2.243
S.D.	±0.191	±0.119	±0.148
20	3.215	1.046	2.705
S.D.	±0.151	±0.115	±0.157
24	3.254	1.292	3.167
S.D.	±0.121	±0.112	±0.156

Eceived: June 1, 2011. Accepted: September 1, 2011. Published: November 1, 2011.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: en trámite

Vol.1 (1): 20-26

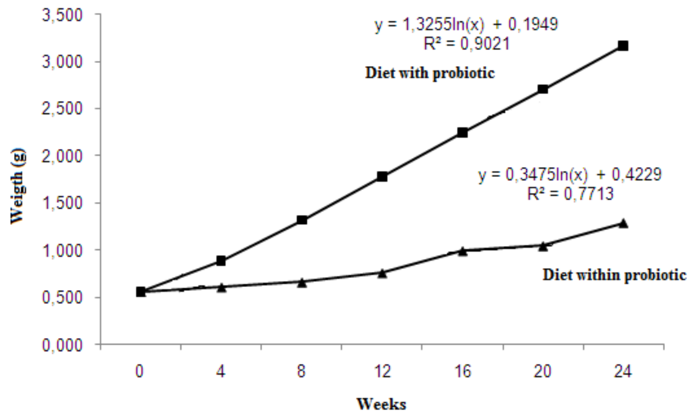

Fig. 1.- Values of the growth curve with respect to the weight of the *C. montezumae* fed with two experimental diets.

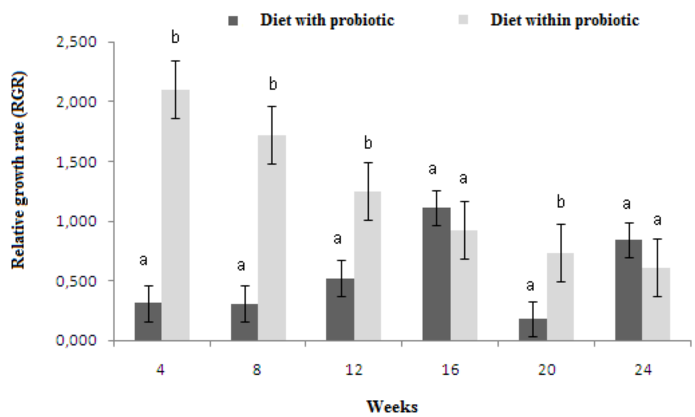

Fig.2.- Relative growth rate (RGR) values with respect to the weight of the *C. montezumae* fed with the two experimental diets. Same letters in the week indicate no significant differences (p<0.05).

DISCUSSION

The elaboration of this food was selected according to the nourishing habits of crayfish, as they have been classified as detritivorus (Avault and Brusnon 1990), herbivore, (Iheu and Bernardo 1993); of opportunist habits (DÁbramo and Robinson 1989) and omnivorous (Rodríguez 1999, Villareal 1991, Wiernicki 1984). It is known that Cambaroides fed at the bottom due to their inability to swim impedes them to capture agile prey. In addition, as other crustaceans, they present a cannibalistic behavior, which is closely related to the season of the year, the age and the contribution of animal protein to the diet (Guan and Wiles 1998, Marshall and Orr 1960, Rodríguez and Carmona 2002). It is for this reason that the quality of the food used in this experiment was adequate in order to obtain a better growth in the organisms, but above all confirm that the addition of a probiotic to the diet permits to accelerate the growth rate.

In general, it has been considered that the use of artificial diets, as the only nutritional feeding source of aquatic microorganism, gives results under the optimum, consequently causing a decrease not only in the growth rate (size), but also in weight, as well as other characteristics such as

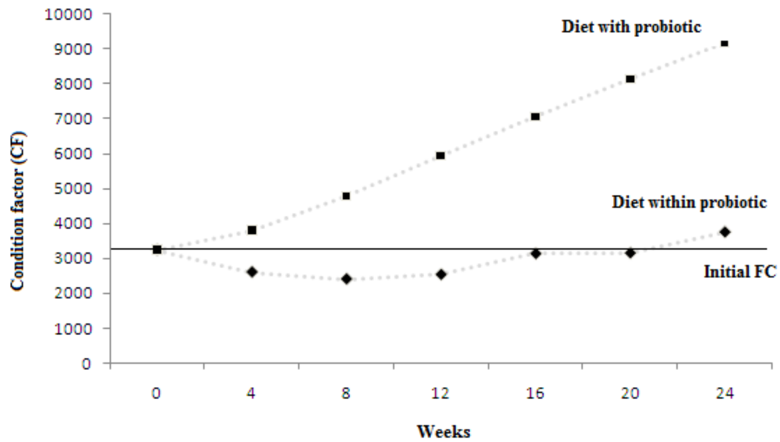

Fig.3.- Condition factor values (FC) of *C. montezumae* fed with the two experimental diets.

pigmentation of the exoskeleton. On the other hand, when diets that present adequate stability in water are integrated it allows these organisms to have an adequate growth, as well as the conditions of the culture media (Jussila 1997). It could be observed in the stability of the diet provided by the bacteriological agar, which impedes the disintegration of the material in the water and consequently losing the nutritional content of the ingredients incorporated, but mainly the lactobacillus employed and its incorporation to the digestive tract of animals.

Another effect of the stability of the diet in water allowed it to be incorporated according to free demand, which was only added when organisms had finished all the food. Compared to other experiments where the supply of food portions is used, which may be low, and the effect thereof upon the growth of organisms is poorly reflected (Clifford 1994, Cortés 1998, Cortés *et al*. 2004, Jussila 1997, Mills *et al.* 1994, O´Brien 1994), as well as a better maintenance of the culture system (Cortés *et al*. 2004, Jussila 1995).

In relation to temperature, it was maintained according to what various authors have mentioned (Jussila 1997, Holdich and Lowery 1998, Mills *et al*. 1994, Morrissy 1992, Morrissy 1990), which is 26ºC and a minimum of 20-23ºC. Variations in temperature may cause stress in these organisms and a diminution in their growth, as these organisms are poikilothermal. That is the reason why variations of temperature should not fluctuate beyond 3ºC. Winberg (1956); Fry (1971), Díaz *et al.* (2006) mention that the temperature is a guideline factor that controls processes such as the activity, consumption of food and growth of poikilotherms. Therefore, keeping cultures without fluctuations will allow a better use of the energy of the diet for such processes. Given the foregoing, a recommendation could be for external cultures, recipients should be buried in order to avoid wide temperature variations (Jussila 1995, Jussila 1997).

Another factor to consider is the density of the organisms, due to concentrations of ammonium in water, which may affect the growth of organisms. Ackefors *et al.* (1994) mentions that densities over 25 org m^2 do not accumulate high concentrations of ammonium, therefore a 50% water replacement is sufficient to maintain levels low. The same happens with dissolved oxygen (Ackefors and Lindqvist 1994) and the pH (Aiken and Waddy 1992, Jussila 1997).

With respect to survival, the use of lactobacillus in the diet allowed it to maintain over 90% (Monroy *et al*. 2009). Mortality found could be the result of stress in the organisms, with the handling of measuring the size and weight every week. It is important to mention that with the non-

Eceived: June 1, 2011. Accepted: September 1, 2011. Published: November 1, 2011.

enriched diet, mortality was over 40%. This coincides with the data of Cortés *et al*. (2004), although in this work, it is also related the mortality found in the molt process, which did not occur in this experiment. Montemayor *et al*. (2010) presents survival values in *P. regiomontanus* from 50-70% compared to what was found with our 90% values with the diet enriched with lactobacillus. In both cases, diets with animal protein were used, Montemayor *et al*. (2010), with squid and ours with shrimp, but additionally enriched with a probiotic. It is important to mention the difference regarding the weight gain presented in the work of Montemayor *et al*. (2010), although they work with *P. clarkii* and *P. regiomontanus*, organisms that reach larger sizes and therefore greater weights. Weight increase is 1374 – 2333 % more, while in *C. montezumae* is 645%. The gain in weight per day is 0.05 and 0.04 g/day in *P. clarkii* and *P. regiomontanus* and in *C. montezumae* is 0.01 g/day.

CONCLUSIONS

We can assure that the use of lactobacillus, in spite that it is a diet enriched with animal and vegetal protein, which can supply the necessary requirements to crayfish, allows accelerating the growth rate of organisms and maintain a weight-size relation (degree of wellbeing) better than animals in culturing.

BIBLIOGRAPHY

Ackefors H, Huner JV, Konikoff M. 1994. Introduction to the general principles of aquaculture. New York, USA. Food Production Press.

Ackefors H, Lindqvist OV. 1994. Cultivation of freshwater crayfishes in Europe. 157-216. En: Huner JV. Ed. Freshwater crayfish aquaculture in North America, Europe, and Australia. Families Astacidae, Cambaridae, and parastacidae. New York, USA. Food Product Press.

Aiken DE, Waddy SL. 1992. The growth process in crayfish. *Reviews in Aquatic Sciences* 6: 335-381.

Avault JW, Brusnon MW. 1990. Crawfish forage and feeding systems. *Reviews in Aquatic Sciences* 3(1): 1-10.

Castro MJ, Espindola RY, Castro MG, Cremiux GJC. 2009. Efectos de dos dosis proteicas en el crecimiento y sobrevivencia de prejuveniles de *Atractosteus tropicus* Gill, 1863 (Pejelagarto). *Biocyt* 2(8):77-88.

Castro BT, De Lara AR, Castro MG, Castro MJ, Malpica SA. 2003. Alimento vivo en la acuicultura. *Contactos* 48: 17-33.

Clifford HC. 1994. El manejo de estanques camaroneros. En: Zendejas H. ed. Memorias del seminario internacional de camaronicultura en México. Camarón '94 Ralston-Purina, Mazatlán, Sin. México: 1-39.

Cortés EJ. 1998. Frecuencia y distribución alimenticia en el cultivo intensivo de juveniles de camarón blanco *Penaeus vannamei.* (Tesis Magister). Centro Interdisciplinario de Ciencias Marinas. Instituto Politécnico Nacional. México. 96 p.

Cortés JE, Villareal CH, Rendón RM. 2004. Efecto de la frecuencia alimenticia en el crecimiento y sobrevivencia de juveniles de langosta de agua dulce *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda:Parastacidae). *Hidrobiológica* 13(002): 151-158.

D'Abramo RL, Robinson EH. 1989. Nutrition of crayfish. *Reviews in Aquatic Sciences* 1(4): 711-728.

Díaz F, Escalante G, Re AD, Sierra E. 2006. Fisiología energética de *Cherax quadricarinatus* (von Martens) alimentado con dos dietas, expuesto a un régimen constante y fluctuante de temperatura. *Hidrobiológica* 16(1): 35-44.

Fry FEJ. 1971. The effects of environmental factors on the physiology of fish. En: Hoar WS, Randal DJ eds. Fish Physiology Vol.VI: Environmental relations and behavior. Academic Press, New York. 1-98 p.

Guan R., Wiles PR. 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusulus* in the Britsh Iowland river. *Aquaculture* 169: 177-193.

Holdich DM, Lowery RS. 1998. Freshwater crayfish: biology, management and exploitation. United Kingdom: Timber Press. 114-144 p.

Iheu M, Bernardo JM. 1993. Experimental evaluation of food preferences of red swamp crayfish, *Procambarus clarkii*: vegetable versus animal. *Freshwater crayfish* 9: 359-364.

Jussila J. 1995. Notes on marron response to high temperature stress. ACWA News. 27-29 p.

Jussila J. 1997. Physiological responses of Astacid and Parastacid crayfishes (Crustacea:Decapoda) to conditions of intensive culture. Perth, Western Australia. Kuopio University Publications C. Natural and Environmental Sciences.136 p.

Kachigan SK. 1991. Multivariate statistical analysis. A conceptual introduction. New York, EUA. Radius Press. 303 p.

Latournerié CJR, Nacif OY, Cárdenas RJ, Romero JJ. 2006. Crecimiento, Producción y Eficiencia de

Energía de Crías de Acocil *Cambarellus montezumae* (Saussure) Alimentas con Detritus de Energía Densa. *REDVET* 7 (12): http://www.veterinaria.org/revistas/redvet/n121206.htm.

Marshall SM, Orr AP. 1960. Feeding and nutrition. En: Waterman TH. ed. The physiology of crustacea, Vol . Metabolism and growth. Academic Press, New York. 227-247 p.

Medina-García M. 1979. El Factor de Condición Múltiple (KM) y su importancia en el Manejo de la Carpa Israle (*Cyprinus carpio)* I. Hembra en el estado de madurez V (Nikolsy, 1963). Manuales Técnicos de Acuacultura. Departamento de Pesca. México. 6p.

Mills BJ, Morrissy NM, Huner JV. 1994. Cultivation of freshwater crayfish in Australia. 217-291. En: Hunner JV. Ed. Freshwater crayfish aquaculture in North America, Europe, and Australia. Families Astacidae, Cambaridae, and Parastacidae. Food Products Press. New York.

Monroy DC, Castro BT, Fernández PFJ, Mayorga RL. 2009. Revisión bibliográfica: Bacteriocinas producidas por bacterias probióticas. *Contactos* 73: 63-72.

Montemayor LJ, Mendoza AR, Aguilera GC, Rodríguez AG. 2010. Influencia de la alimentación sobre la reproducción y el crecimiento del acocil regio (*Procambarus regiomontanus*), especie en peligro de extinción. *Ciencia UANL* 13(3): 276-286.

Morrissy NM. 1990. Optimum and favorable temperatures for growth of *Cherax tenuimanus* (Smith 1912) (Decapoda:Parastacidae). *Australian J Mar Freshwater Res.* 41: 735-746.

Morrissy NM. 1992. An introduction to marron and other freshwater crayfish farming in Western Australia. Fisheries Department of Western Australia.

O'Brien B. 1994. Feeding marron. *Marron Grower's Bulletin* 16(4): 14-15.

Rodríguez SM, Carmona OC. 2002. Balance energético del Acocil *Cambarellus montezumae* (Saussure) (Crustáceo: ASTACIDAE: CAMBARIDE) perdida de energía en la tasa metabólica. *Universidad y Ciencia* 18 (036): 128-134.

Rodríguez SM. 1999. Biología y sistemática de los Cambáridos del Sudeste de México y su potencial aprovechamiento en la acuicultura. (Tesis Bachiller). Biología: Universidad Autónoma Metropolitana-Xochimilco. 118 p.

Sokal RR, Rohlf JF. 1981. Biometry. The principles and practice of statistics in biological research. 2a ed. San Francisco, California, USA. WH Freeman. 859 p.

Tatsuoka MM. 1970. Selected topics in advanced statistics. And elementary approach. Discriminant analysis. Illinios, EUA. IPAT, 6:1-57.

Villareal H. A partial energy budget for the Australian crayfish *Cherax tenuimanus*. Journal of the World Aquaculture Society. 1991; 22: 252-259.

Wiernicki C. 1984. Assimilation efficiency by *Procambarus clarkii* fed elodea (*Egeria densa*) and its products of decomposition. *Aquaculture* 36: 203-215.

Winberg GG. 1956. The rate of metabolism and food requirements of fishes. Belorussian University, Minsk. In Russian (English translation: Fisheries Research Board of Canada Translation Series). 194 p.

Eceived: June 1, 2011. Accepted: September 1, 2011. Published: November 1, 2011.