Pak. J. Bot., 42(6): 4199-4206, 2010.

EFFICACY OF FOOD GRADE LACTIC ACID PRODUCED THROUGH BACTERIAL FERMENTATION

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Abstract

The efficacy studies for lactic acid were conducted using rats to observe the toxicity of food grade lactic acid, produced in laboratory through fermentation process. The Sprague-Dawley rats were used for the evaluation of food grade lactic acid and was given in the feed to five groups of rats (A-E) at the dose of 0, 1, 2, 3 and 4%, respectively. The behavioral changes, feed intake, body weight and hepatocytic changes were recorded during the experiment for 28 days. Incorporation of lactic acid in the diets at these concentrations did not manifest a significant ($p<0.05$) effect on behavior, feed intake and body weight in all the rats groups tested for lactic acid toxicity. Liver weight, relative liver weight, cell diameter and nucleus diameter remained unchanged ($p<0.05$) on ingestion of lactic acid suggesting that the consumption of lactic acid up to 4% is safe in the rats. However, these results are yet to be validated in the humans as far as the use of lactic acid produced through bacterial fermentation in the food is concerned.

Introduction

The corn cobs contain significant amount of cellulosic material, which may be the best source of fermentable sugars for the production of organic acids i.e. lactic acid. The sludge containing high amount of cellulose can be hydrolyzed to glucose by the use of cellulase enzymes before treating with lactic acid bacteria. The bioconversion of cellulosic material to lactic acid can be accomplished by carrying out simultaneous sacchrification and fermentation at optimum pH and temperature around 5 and 40^{o}C respectively (Nakasaki & Adachi, 2003).

There are two types of bacteria that have the ability to produce lactic acid from different food substrates. *Lactobacillus bulgaricus* being a useful microorganisms is used to ferment milk with lactic acid for the production of yoghurt (Khan *et al*., 2008). Similarly,the homofermentatitive *Lactobacillus delbruekii* bacteria are used to produce only lactic acid from cellulose and hetrofermentatative *Lactobacillus acidophilus* produces other products in addition to lactic acid. The lactic acid produced, strongly inhibits other microbial activity in lactic acid fermentation. It further restricts cellulase activity but less severely than the inhibition of microbial activity because a lactic acid content of > 90 g/liter is needed for 50% inhibition. A gradual deterioration of the simultaneous saccharification and fermentation process occurs with the build-up of lactic acid and the rate limiting step in simultaneous saccharification and fermentation shifts from hydrolysis to fermentation as the bioprocess proceeds (Lyer & Lee 1999).

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The lactic acid is widely used in food industries. It exhibits preservative properties; inhibiting microbial spoilage in meats, sea foods, mayonnaise, salad dressing and soft drinks. The emulsifiers derived from lactic acid improve the quality of breads, cake mixes, filling and toppings, powdered coffee, shortenings and whiteners. Lactic acid also acts as a flavor enhancer in beer, wine cider, soft drinks, candies, frozen desserts, jams and jellies, margarine and pickles (Oda *et al*., 1997). Lactic acid, present in many foods, both naturally or as product of microbial fermentation is used in food and food applications as an acidifier, curing agent and pH control agent (Andres, 1985).

Toxicity of various organic acids has only been demonstrated at very high doses e.g. signs of acute toxicity in rats following ingestion of 11.7 g/kg body weight citric acid indicated motor ataxia, mydriasis (abnormal pupil dilation) and decreased rate of respiration and heart beat (Yokotani *et al*., 1971).

The objective of the present study was to assess the toxicity of food grade lactic acid, produced through bacterial fermentation. Behavioral changes, feed intake, body weight and liver weight were used as indices to assess the effect of feeding various levels of lactic acid to rats.

Materials and Methods

Housing and maintenance of animals: Forty adult male albino rats with an initial weight of 160g were procured from National Institute of Health (Veterinary Division), Islamabad, Pakistan and were divided into five groups (A-E) comprising eight rats in each group. The animals were housed singly in wire bottomed cages and maintained in a temperature-controlled room at 25 ± 2 ºC under 12 hours light/dark cycle. The feed and water were given *ad libitum*. The rats were kept for one week on the basal diet before feeding experiment diet.

Administration of lactic acid to rats: The lactic acid produced from corn cob hydrolysate was mixed in the feed. The five experimental diets were prepared by mixing lactic acid @ 0, 1, 2, 3 and 4% in the test diets i.e., D_0, D_1, D_2, D_3 and D_4 respectively. These diets were given to rats of group A, B, C, D, and E for a period of four weeks. The composition of the standard diet is given in Table 1. The diets of the rats were prepared following the standards outlined in AIN-93 with a little modification (Reeves *et al*., 1993).

Behavioral observations: The animals were observed twice daily for clinical signs exhibited by them in different groups. The behavioral alterations were observed subjectively by observing alertness, attraction to feed and water responsiveness to external stimulus and activity.

Feed intake and body weight: Body weight was measured on weekly basis while feed consumption was noted daily for the whole experimental period. Data were recorded for change in body weight and feed consumption.

Necropsy and histopathology: These tests were performed by following the method of Lille *et al*., (1976). The animals were sacrificed at the end of study period. The liver was weighed and its relative weight was also calculated as a function of body weight.

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Table 1. Compositions of diets (g/kg) fed to the male rats.

Ingredients	Composition	R* AIN-93
Flour (750)	585 (Starch)	~600
Casein (85)	140 (Protein)	140
Canola oil (20)	40 (Fat)	40
Potato starch (35)	50 (Fiber)	50
Sucrose (75)	75 (Sucrose)	100
Mineral mix (15)	15 (Min. mix)	35

R* = Recommendation, based on AIN-93M diet of adult rodents.

The liver was fixed in 10% neutral buffer formalin and processed for histopathological examination using routine method of dehydration in ascending series of ethyl alcohol, clearing in xylene, impregnation and embedding in paraffin. The sections of 5 µm thickness were cut and stained with hematoxylin and eosin. The stained sections were examined for morphological alterations and morphometric measurements. The diameter of hepatocytic cytoplasm and nucleus were recorded by use of calibrated ocular micrometer.

Statistical analysis: Data obtained were tabulated and subjected to statistical analysis using analysis of variance and Completely Randomized Design (CRD) (Steel *et al*., 1997). Duncan's Multiple Range Test was applied to assess the difference between means (Duncan, 1955). The data represent the mean of eight values i.e. no. of rats in each treatment group. Statistical significance was set at $p \leq 0.05$ probability levels.

Results and Discussion

Behavioral alterations and clinical signs: No behavioral change was observed in rats for alertness, attraction to feed and water, responsiveness to external stimulus and activity in all the groups of rats. All the rats in each group exhibited normal behavior and remained active. All the rats showed attraction towards feed and water. The rats remained active and were alert upon tapping of the cage wall. However, rats fed 4% lactic acid showed mild depression when the responses were checked physically against the stimulus and other activities during the last week of the trial. These rats further exhibited diarrhoea characterized by pasting of fecal matter at 25th day of the study. Nevertheless, no such signs of diarrhoea were observed in other groups of rats. The livers of the rats were of normal color, size, shape and consistency in all groups and no pathological lesions were observed.

Feed intake: Feeding time exerted a significant effect ($p<0.05$) on the feed intake of the rats and a concomitant increase in feed intake over the entire feeding time was observed however, when the observation was made with respect to the levels of lactic acid in the diet, the use of lactic acid showed non significant effect ($p<0.05$) on the feed intake of rats. The feed consumption on an average varied from 86.37 to 108.21g/week during four weeks of the study (Fig. 1A,B). However, the feed intake was relatively higher in control as compared to the lactic acid administered group of rats though the difference was non significant ($p<0.05$).

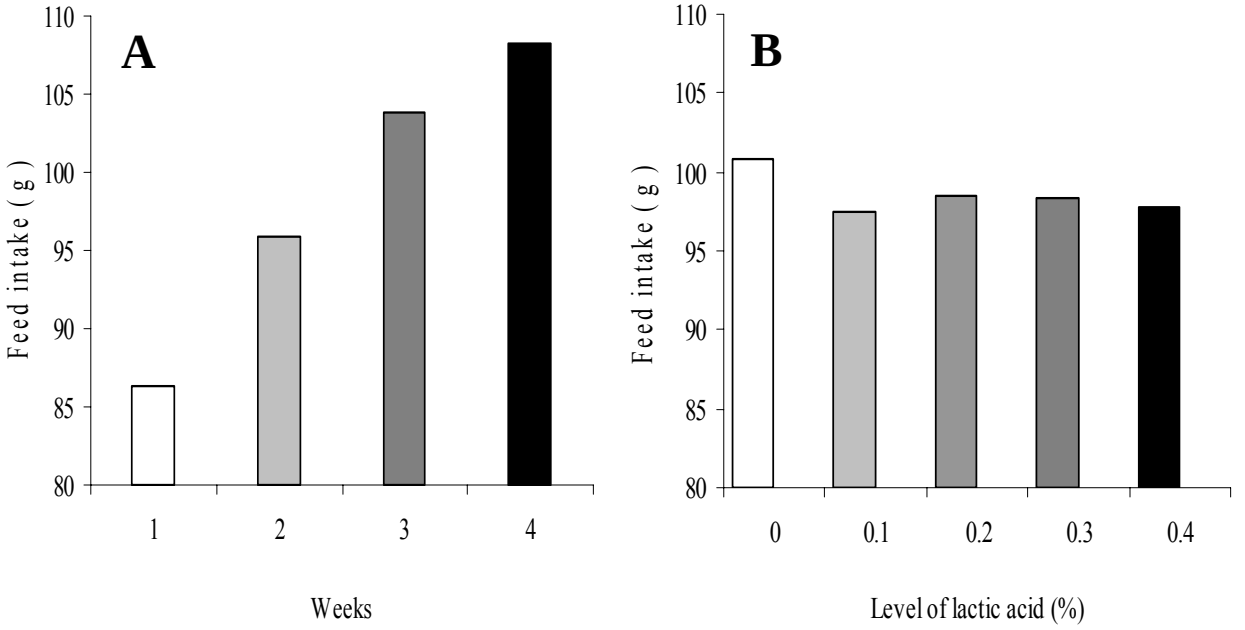

Fig. 1. (A) Effect of lactic acid on the feed intake of rats fed lactic acid supplemented feed for a period of four weeks. (B) Variability in feed intake of rats fed various concentrations of lactic acid in the feed. The values indicate the means of the two experiments, n= 8 (n is the No. of rats in each groups). The bars bearing ** are significantly ($p<0.05$) different from control.

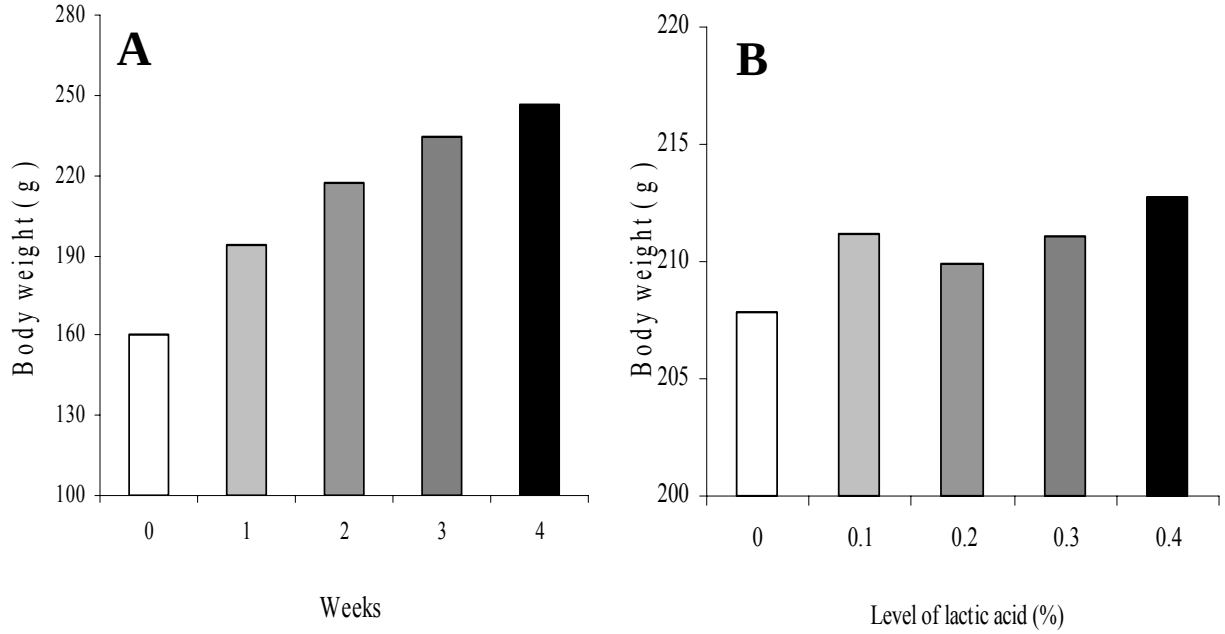

Fig. 2. (A) Effect of lactic acid on body weight of rats fed lactic acid supplemented feed for a period of four weeks. (B) Variability in body weight of rats fed various concentrations of lactic acid in the feed. The values indicate the means of the two experiments, n= 8 (n is the No. of rats in each groups). The bars bearing ** are significantly ($p<0.05$) different from control.

Body weight: The body weight of rats fed lactic acid supplemented diet has been given in Figure 2. The data manifested that body weight was significantly higher at 4^{th}. week of feeding and body weight was significantly lower at the start of the experiment. There was a concomitant increase in the body weight of the rats during the entire feeding period. The body weight of rats ranged from 160.69 to 246.39g between 0 and 4th week of feed intake respectively (Fig. 2A).

The effect of lactic acid was found to be non significant on the body weight of rats (Fig. 2B). The rats which were fed lactic acid supplemented diet gained relatively higher overall body weight than the control rats albeit these differences remain minimal.

Liver weight, Relative liver weight, Liver cell diameter (µm) and Hepatocytic nucleus diameter (µm): It is obvious from the statistical results presented in Table 2 that liver weight was not primarily affected on ingestion of lactic acid by the rats. The average liver weight ranged from 4.89 to 5.00g among different groups of rats. Similarly, there was a slight increase in the liver weight with an increment in the level of lactic acid in the feed, yet the change was statistically non significant ($p<0.05$). The liver weight was recorded to be maximum in the rats of group fed 4% lactic acid (5.00g) and minimum in control group i.e., 4.92g (Table 2). A similar pattern was observed in relative organ weight and liver cell diameter of the experimental rats. Supplementation of lactic acid seemed likely having no effect ($p<0.05$) on these indices (Table 2). The liver cell diameter ranged from 14.76 to 15.10µm among different groups of the experimental rats. The cell diameter increased up to 15.10µm in group fed 4% lactic acid and the cell diameter was 14.76µm in control.

The diameter of nucleus ranged from 7.66 to 8.00µm among different groups of experimental rats (Table 2). The differences among various groups for nucleus diameter were found to be non significant ($p<0.05$). However, the cell nucleus diameter was lower in the rats fed control diet as compared to the groups of rats fed with lactic acid supplemented diet.

Discussion

Growth is primarily considered as an index to assess adverse effects of supplementing the feed with various additives. Feed intake and body weight increased concurrently with an increase in experimentation time. Incorporation of lactic acid in the feed did not show any injurious effect in the rats suggesting that the use of lactic acid in the feed was safe. The results obtained in the present study (Fig. 2 A,B) revealed that lactic acid administration up to the concentration of 4% was unable to pose any health risk as far as the growth of the animals was concerned. Furthermore, no adverse behavioral changes and clinical signs in rats were recorded during the course of the experiment. The difference in liver weight, hepatocytic diameter and nucleus diameter also remained non significant with the supplementation of lactic acid in the diet of rats. The range of liver cell and nucleus diameter were not affected in size with the administration of lactic acid (Fig. 3A-D). Previous studies conducted on the efficacy of lactic acid on animals also revealed that lactic acid had no adverse effect on the health of the experimental animals. Other researchers investigated the general safety of immune-enhancing lactic acid bacteria (LAB) strains *Lactobacillus rhamnosus* HN001 (DR20(TM)), Lb. *acidophilus* HN017, and *Bifidobacterium lactis* in a feeding trial. The results demonstrated that 4 weeks consumption of these strains had no adverse effects on animals' general health status, hematology, blood biochemistry, gut mucosal histology parameters, or the incidence of bacterial translocation. The results obtained in this study suggested that the potentially probiotic LAB strains HN001, HN017, and HN019 are non-toxic for mice and are therefore likely to be safe for human use (Zhou *et al*., 2000). Photomicrographs of liver cell of albino rats fed lactic acid (0-4%) showed that the liver tissues were not affected and stayed normal (Fig. 3A-D).

Table 2. Mean values for liver weight, relative liver weight, cell diameter and nucleus diameter of rats fed lactic acid.

Lactic acid (%)	*LW (g)	♦RLW	•CD (µm)	δND (µm)
0	4.92[a]	2.02[a]	14.76[a]	7.66[a]
1	4.99[a]	2.02[a]	14.85[a]	7.72[a]
2	4.89[a]	2.00[a]	14.88[a]	7.78[a]
3	4.95[a]	2.00[a]	14.98[a]	7.87[a]
4	5.00[a]	2.00[a]	15.10[a]	8.00[a]

The means carrying same letters in a column and row are not significantly different
*Liver weight, ♦Relative liver weight, •Cell diameter, δNucleus diameter

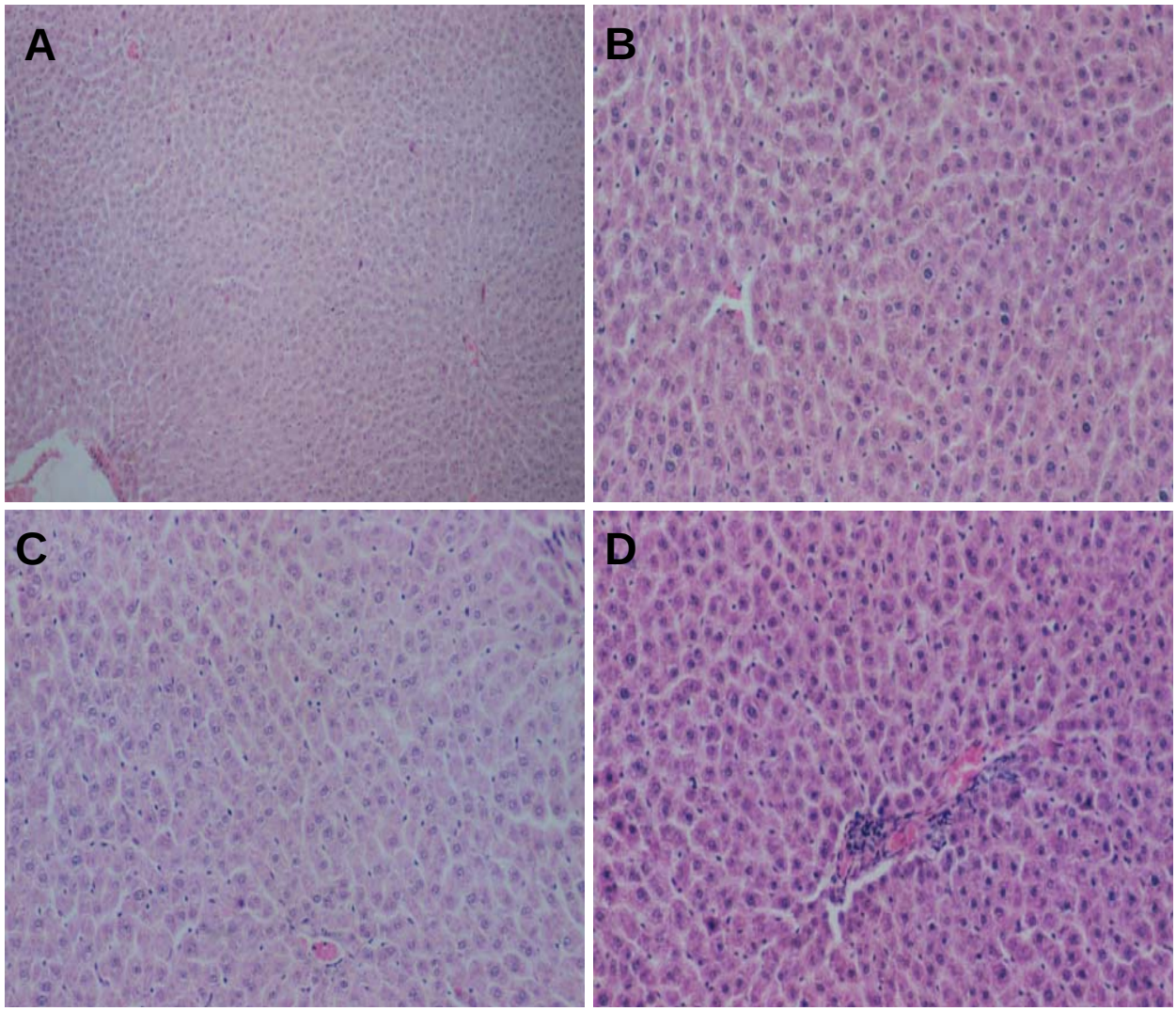

Fig. 3. Photomicrograph of liver cell of albino rats fed lactic acid showing normal behavior at (**A**) (0.1% level at 50x) (**B**) 0.2% at 150x (**C**) 0.3% at 250x (**D**) 0.4% al 300x (H & E Stain).

In another study, the rats readily consumed the ration with 13.4% lactic acid in the dry matter. The test rats showed normal behavior and gait, no signs of muscular stiffness, and the walking tests did not reveal any difference when compared with the control animals. The test rats ingested 1.2 g each of L+ and D – lactic acid daily. They consumed 12 g lactic acid per kg live weight, which is about three times the LD 50 of lactic acid. However, the LD50 of 4 g lactic acid per kg live weight is based on acute toxicity after

oral administration of lactic acid with an unknown percentage of the D–isomer (Smyth *et al*., (1941); Furth & Engel (1930); Anon., 1967) indicating that lactic acid did not accumulate in the body of rats fed with 1 or 2 g sodium lactate per kg body weight for a period of 14–16 days. The rats, in the present experiment were gradually adapted to the ration with pure lactic acid and they tolerated it well for a period of 4 weeks. The results suggest that lactic acid produced in the laboratory is of food grade and can be used for preparation of food products as an acidulant on large scale as no physiological disorder was observed in rats as a result of lactic acid supplementation in the feed.

In rats given doses of lactic acid up to 1.3 g/kg body weight by gastric gavage, signs of toxicity included difficulty breathing, runny nose and abdominal inflation immediately after dosing (Morotomi, 1981).

Probiotics are the live microbial supplements of single or mixed cultures that produce health beneficial effects when ingested (Hussain *et al*., 2008).Animal studies on the potential use of *L. acidophilus* LAP5 as human and animal probiotics confirmed no toxic effects by dietary administration at concentrations ranging $2.1x10^{11}$ CFU per kg body weight of rat per day for 28 days. The researchers evaluated the toxicity of strain *L. acidophilus* LAP5 in Wistar rats and there were no adverse effects on the general conditions and behavior, growth, feed and water consumption, hematology, clinical chemistry indices, organ weights and histopathologic analysis (Cheng *et al*., 2004) suggesting that consumption of strain *L. acidophilus* LAP5 was not associated with any signs of toxicity in Wister rats even following consumption of large quantities.

Similarly, in another study, there were no differences observed in insulin, blood lipids or liver cholesterol between rats groups fed diets where lactic acid was added after baking or with addition of probiotic bacteria. The authors however, confirmed that bread baked in the presence of lactic acid improved glucose metabolism in obese and hyperinsulinaemic Zucker rats (Ostmana *et al*., 2005).

The research suggested that lactic acid produced in the laboratory is of food grade and can be used for preparation of bread as an acidulant on large scale.Animals treated with lactic acid did not show any behavioral and physiological abnormality during the entire experimental period.

Acknowledgement

The authors greatly acknowledge research facilities provided by National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan to successfully complete this project

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(Received for publication 21 January 2009)