JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Animal Feed Enriched with Se and Clays on Hg Bioaccumulation in Chickens: In Vivo Experimental Study

ANA I. CABAÑERO, YOLANDA MADRID, AND CARMEN CÁMARA*

Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain

An in vivo experiment was conducted to evaluate the effects of sodium selenite, sepiolite, and bentonite on inorganic mercury (Hg) and methylmercury (MeHg) bioaccumulation. For this purpose 160 chickens were fed under different controlled conditions. Chickens were exposed to Hg(II) and MeHg added to feed with or without selenium or clays supplementation. No significant differences were observed in the voluntary intake and feed/gain conversion rates. The target organs of Hg(II) and MeHg in chickens were the liver and kidney, respectively, but the greatest body store was the muscle in both cases. A higher bioaccumulation for MeHg than for Hg(II) was observed. The results showed that addition of sodium selenite, sepiolite, or bentonite induced a decrease of up to 60−100% in the inorganic mercury bioabsorption. Bentonite addition to a MeHg-containing diet also caused a decrease in organic mercury bioaccumulation (29−67%). On the other hand, inorganic selenium and sepiolite did not decrease MeHg accumulation.

KEYWORDS: Selenium; mercury; bioaccumulation; chicken; animal feed; clays

INTRODUCTION

Mercury (Hg) is a widespread and persistent pollutant in the environment and is among the most highly bioconcentrated trace metals in the human food chain. Mercury toxicity, bioavailability, and environmental mobility are well-known to be highly dependent on its chemical form (*1*). Organic mercury compounds are of special concern because of their easier penetration through biological membranes, more efficient bioaccumulation, higher stability, and slower elimination from tissues (*2*) compared to inorganic mercury, and methylmercury (MeHg) is one of the most important Hg species in terms of bioaccumulation and risk.

Selenium (Se) is an essential micronutrient for humans, a constituent of enzymes, and is also well-known for its potential in disease prevention (*3*). Several authors consider that selenium can act as a potential antagonist of mercury toxicity (*4*, *5*), but the way in which it interferes with mercury is still uncertain; several mechanisms have been proposed to explain this interaction, although none of them is conclusive (*6*). Some of the more likely hypotheses are that Se may promote a redistribution of Hg from more sensitive organs (kidney, central nervous system) to less sensitive ones (muscle), that there is competition of Se for the same receptors, that complexes, such as tiemannite (*7*) or Se−Hg−S species, are formed (*8*) and that MeHg conversion into less toxic forms is promoted and oxidative damage prevented (*9*).

During the past decade some contamination events of animal feed in Europe, such as a dioxin contamination episode in Belgium in May 1999, have been detected. Therefore, measures have been introduced by the European Union to protect and improve the quality of human health (*10*).

Nowadays, fish meal is used as a source of protein in feed for poultry and swine (*11*) in Europe. Poultry, swine, and fish fed these meals could concentrate mercury to undesirable levels if care is not taken (*12*). Even low levels of fish meals containing mercury fed to swine and poultry can cause mercury accumulation in the flesh exceeding 0.03 mg kg^{-1}, the maximum residue limit (MRL) in most countries for nonfish food stuffs (*12*). Actually, experiments in feeding fish meal to poultry have shown that tissue mercury accumulation correlates with the mercury concentration of the meal (*13*).

Therefore, meat from animals fed fish meal or other fish products is likely to contribute to the exposure to mercury (*11*). In fact, such exposure could explain previous findings of unexpectedly high MeHg levels found in individuals with low fish consumption and a possible influence of chicken consumption on the concentration of MeHg in human umbilical cord blood (*13*).

Removing Hg effects from contaminated foodstuffs remains a major problem. One approach could be to use non-nutritive adsorbing materials in the diet in order to bind Hg and reduce its absorption from the gastrointestinal tract or the use of agents that could act as antidotes or antagonize the toxic effect of Hg(II) and MeHg. Non-nutritive adsorbing materials such as cation exchangers (e.g., zeolites) have been previously used in the diet to bind Hg and reduce its absorption from the gastrointestinal tract (*14*). However, no data about the use of other non-nutritive adsorbing material such as clays has been published.

Clays are characterized by their micrometer-sized particles, swelling properties, large surface areas, high cation exchange

* Author to whom correspondence should be addressed (e-mail ccamara@quim.ucm.es).

10.1021/jf048267v CCC: $30.25
Published on Web 02/19/2005

Table 1. Experimental Setup

treatment	no. of pens	total no. of chickens
basal diet		
control	1	8
+ selenium (0.2 mg kg^{-1} in feed)	1	8
+ bentonite (2 g kg^{-1} in feed)	1	8
+ sepiolite (2 g kg^{-1} in feed)	1	8
basal diet + Hg(II) [0.2 mg kg^{-1} in feed]		
control	2	16
+ selenium (0.2 mg kg^{-1} in feed)	2	16
+ bentonite (2 g kg^{-1} in feed)	2	16
+ sepiolite (2 g kg^{-1} in feed)	2	16
basal diet + MeHg [0.2 mg kg^{-1} in feed]		
control	2	16
+ selenium (0.2 mg kg^{-1} in feed)	2	16
+ bentonite (2 g kg^{-1} in feed)	2	16
+ sepiolite (2 g kg^{-1} in feed)	2	16
total	20	160

capacity, chemical stability, and charge distribution. On the basis of their physical and chemical properties, these products are widely employed as additives (binders, anticaking agents, coagulants, lubricants, or agglomerate) in foodstuffs, although they are generally present as a minor component depending on the established regulation of each country (1−2% w w^{-1}) (*10*). Recently a role as enhancer of the nutritive value of diets in ruminants and monogastric animals has been also proposed (*15*).

The special characteristics of sepiolite make it ideal for use as an adsorbent for toxins, bacteria, and even viruses in the intestine (*16*), as pharmaceutical excipients, or as active ingredients (*17*), as well as lubricants of ground diets and as a pelleting agent during feed processing procedures. With these characteristics, it might promote significant modifications in the physical and chemical properties of digesta contents (*15*).

Bentonite is used as an animal feed supplement and as a pelletizing aid in the production of the animal feed pellets, as well as a flowability aid for unconsolidated feed ingredients such as soy meal. It is also used in the removal of impurities in oils and as a clarification agent in drinks such as beer, wine, and mineral water. It also has been shown to reduce the toxic effect of aflatoxins contained in fish food (*18*).

In this work several studies have been performed to evaluate, on the one hand, the mercury distribution and possible modifications in naturally occurring levels of Se and, on the other, the effect of Se, bentonite, and sepiolite administration on mercury distribution and bioaccumulation in broiler chickens.

EXPERIMENTAL PROCEDURES

Animals, Diets, and Experimental Setup. One hundred and sixty 1-day-old Hybro-G female broiler chickens were used in this study. The birds were randomly assigned into 20 pens for treatment, each of 8 birds. All pens were bedded with a wood-shavings litter and equipped with feeders and waterers in an environmental chamber with 37.5 cm^2 per bird.

The chickens (during a study period of 42 days) were fed either with a common basal diet (control), formulated to contain all nutrients required, or with a diet supplemented with different compounds [Hg(II), MeHg, Se(IV), sepiolite, or bentonite] specified in **Table 1**. Ingredients and chemical composition of the basal diet are shown in **Table 2**.

The diets and fresh water were offered ad libitum. Lights were on for 24 h during the first 3 days, after which a lighting schedule was applied consisting of 20 h of light and 4 h of darkness. The light intensity was reduced gradually during the experiment.

Table 2. Composition of the Basal Diet Used in the Experiments

ingredient	0–42 days (%)
barley	5.000
wheat	30.000
maize	18.568
soyabean	35.903
soja oil	6.453
calcium carbonate	0.564
dicalcium phosphate	2.267
sodium chloride	0.299
sodium carbonate	0.186
DL-methionine	0.159
Avizyme 1300	0.100
SV-5211-MxMa	0.500
total	100.000
analysis	
true metabolizable energy (kcal/kg)	3075
dry matter	88.55
PB	22.18
EE	8.50
FB	2.63
ash	6.28
carbohydrates	32.57
sugars	4.64
calcium	0.91
phosphorus	0.75
available phosphorus	0.45
Cl	0.22
sodium	0.18
lysine	1.23
methionine	0.55
methionine + cystine	0.93
Thr	0.84
Trp	0.28
lysine av	1.07
methionine + cystine av	0.82
Thr av	0.69
Trp av	0.24
LI-C18:2	4.60
Na + KCl	260
unsaturated	6.95
saturated	1.20

The chickens were weighed at 0, 21, and 42 days of age to determine gains in body weight and feed efficiency. Mortality was recorded as it occurred (**Table 3**). During the experiment, temperature and humidity were registered. These conditions were in accordance with animal welfare.

The experimental design consisted of 12 different dietary treatments (**Table 1**), to evaluate the effect of selenium, sepiolite, and bentonite on mercury bioaccumulation.

At the end of day 42, the experiment was finished. For evaluation of mercury bioaccumulation in the indicated cases, all birds were slaughtered. The carcasses were manually eviscerated, and the liver, skin, kidney, and muscle of each chicken were collected and stored individually at −18 °C. The frozen organs from six animals/group were individually blended and oven-dried at 40 °C for 2 days and stored at −18 °C until analysis.

Instrumentation. An atomic fluorescence spectrometer (AFS, Merlin 10.023, P. S. Analytical Ltd., Orpington, Kent, U.K.) was used to determine the total mercury content. Mercury vapor was generated in a flow injection system using a multichannel peristaltic pump (Gilson, Villiers-le-be, France), a six-way injection valve (Omnifit, Cambridge, U.K.), and a gas−liquid separator. The separator was coupled to a commercial dryer membrane (Perma Pure Products, Farmingdale, NJ) to eliminate the moisture, and both together were used as an interface for CV-AFS.

An atomic fluorescence spectrometer (AFS, Excalibur, P. S. Analytical Ltd.) was used to determine the total selenium content. Selenium hydride was generated in a flow injection system using a peristaltic pump (Gilson) and a gas−liquid separator. The separator was coupled to a commercial dryer membrane (Perma Pure Products) to eliminate the moisture, and both together were used as an interface for HG-AFS.

Table 3. Effect of Hg, MeHg, Se, Bentonite, and Sepiolite on Body Weight Gain and Food Conversion[a]

treatment	bird body wt (g), day 0	bird body wt gain (g), day 21	bird body wt gain (g), day 42	food conversion ratio [g of food (g of gain^{-1})], day 21	food conversion ratio [g of food (g of gain^{-1})], day 42	mortality (%)
basal diet						
control	43 ± 2	733 ± 30	2426 ± 98	1.56 ± 0.05	1.72 ± 0.08	12
+ selenium	42 ± 1	726 ± 41	2389 ± 95	1.68 ± 0.06	1.69 ± 0.07	12
+ bentonite	42 ± 2	752 ± 32	2405 ± 78	1.59 ± 0.06	1.77 ± 0.07	0
+ sepiolite	41 ± 3	769 ± 45	2415 ± 89	1.67 ± 0.07	1.77 ± 0.09	12
basal diet + CH_3ClHg						
control	43 ± 2	795 ± 29	2451 ± 85	1.54 ± 0.05	1.76 ± 0.08	6
+ selenium	42 ± 2	769 ± 42	2359 ± 101	1.50 ± 0.06	1.72 ± 0.08	0
+ bentonite	41 ± 3	775 ± 27	2464 ± 90	1.59 ± 0.07	1.78 ± 0.09	0
+ sepiolite	42 ± 2	758 ± 27	2357 ± 100	1.55 ± 0.06	1.75 ± 0.10	0
basal diet + $HgCl_2$						
control	41 ± 3	749 ± 32	2472 ± 91	1.52 ± 0.05	1.70 ± 0.06	6
+ selenium	42 ± 2	798 ± 41	2462 ± 98	1.52 ± 0.05	1.74 ± 0.08	6
+ bentonite	42 ± 3	802 ± 38	2452 ± 96	1.54 ± 0.07	1.74 ± 0.07	0
+ sepiolite	41 ± 2	774 ± 33	2281 ± 93	1.55 ± 0.07	1.76 ± 0.09	6

[a] Results expressed as mean value ± SD.

For total Hg and Se determination, samples were microwave digested in double-walled advanced composite vessels using an oven with a power of up to 1000 W (MSP, CEM, Matthews, NC).

Reagents. Mercury standards solutions were prepared by appropriate dilution of a stock mercury chloride solution [1000 mg Hg(II) L^{-1}] (Merck, Darmstadt, Germany) and methylmercury chloride [1000 mg MeHg L^{-1}] (Alfa Aesar, Karlsruhe, Germany) in deionized Milli-Q water (Millipore, Bedford, MA). These solutions were stored in amber vials at −18 °C. Standards were prepared daily to reduce mercury losses by volatilization.

Inorganic selenium solution was obtained by dissolving sodium selenite (Merck) in deionized Milli-Q water. Stock solutions of 10 mg Se(IV) L^{-1} were stored in the dark at 4 °C. Working standard solutions were prepared daily by dilution.

Sepiolite (Exal UE-562, Tolsa, S.A., Madrid, Spain) and bentonite (Toxisorb, Lohmann Animal Health GmbH & Co., Cuxhaven, Germany) were added to the basal diet.

Stannous chloride (3% w v^{-1}), used as a reducing agent for Hg(II) in CV-AFS, was prepared by dissolving the appropriate mass of stannous chloride, anhydrous (Merck), in 3 M hydrochloric acid that had been prepared by diluting 12 M hydrochloric acid (Merck) with ultrapure water.

Sodium tetrahydroborate 0.5% (w v^{-1}), used as a reducing agent for Se(IV) in HG-AFS, was prepared by dissolving $NaBH_4$ powder (Merck, Steinheim, Germany) in deionized Milli-Q water, stabilized in 0.15% (w v^{-1}) NaOH, and filtered to eliminate turbidity.

H_2O_2 (35%) from Panreac and HNO_3 (65%) were used for acid digestion of samples.

Argon (purity = 99.999%, Carburos Metálicos, Barcelona, Spain) was used as a makeup gas, sheath gas at the transfer line, and carrier gas with AFS, respectively.

Measurements. *Total Mercury Quantification.* To determine the total mercury content, the dry samples (50−200 mg) were digested with 1−2 mL of concentrated nitric acid and 0.5 mL of 35% hydrogen peroxide in an analytical microwave oven at 43% power output. The pressure was held at 20 psi for 15 min, at 40 psi for 30 min, and finally at 85 psi for 1 h.

Total mercury concentration was determined by both external and standard addition calibrations of the signal obtained by the continuous mercury cold vapor system connected to AFS equipment. A flow rate of 2.5 mL min^{-1} (3 M hydrochloric acid) and a similar flow rate of the reductant solution (3% stannous chloride in 3 M hydrochloric acid) were used to generate the mercury cold vapor.

Total Selenium Quantification. The samples followed the same acid digestion as mentioned for total mercury quantification.

Se(VI) was reduced to Se(IV) by adding concentrated hydrochloric acid (6 M final concentration) to the digest and heating at 95 °C for 1 h. The solutions were then diluted to 25 mL with Milli-Q water.

Total selenium concentration was determined by the continuous selenium hydride system connected to AFS equipment. A flow rate of 1.5 mL min^{-1} (3 M hydrochloric acid) and a similar flow rate of the reductant solution (1% sodium tetrahydroborate w v^{-1}) were used to generate the selenium hydride.

Validation of the Results. In the present work, two certified reference materials were employed for validation of the methodologies used. Method validation for mercury was performed by using the reference material CRM-463 (tuna fish), certified for methylmercury (2.85 ± 0.16 μg g^{-1}), from the Community Bureau of Reference of European Commission (BCR), whereas for total selenium a marine tissue reference material (Murst-ISS A2), certified for total selenium (7.37 ± 0.91 μg g^{-1}) from Institute for Reference Materials and Measurements, was used.

Because, at the 95% confidence level, no significant differences were detected between the certified value and the experimental one [(2.86 ± 0.10 μg of Hg g^{-1}) and (7.41 ± 0.6 μg of Se g^{-1})], the method used was considered to be accurate for total mercury and selenium determination.

Statistical Analysis. A one-factor analysis of variance was applied to detect possible differences in total mercury and selenium between the different treatments studied. A significance level of $P < 0.05$ was adopted for all comparisons. Statgraphics Plus version 4.0 (Statistical Graphics) was used for the statistical analysis.

RESULTS AND DISCUSSION

Feed Intake and Growth. Data presented in **Table 3** show the effect of the 12 dietary treatments on body weight gain and feed conversion ratios of broilers at 0, 21, and 42 days of age. Neither selenium nor clays (sepiolite and bentonite) added to the basal diet had a significant effect on feed conversion. These data agree with the findings of other authors, who reported similar results for sepiolite and bentonite (*15*, *19*−*20*).

Furthermore, the results show that chickens fed with a mercury concentration of 0.2 mg kg^{-1} with or without Se and clays had similar weight gains up to 42 days.

No differences were found in feed conversion among treatments and between groups on the 12 treatments ($P < 0.05$). Therefore, the inclusion of Hg(II), MeHg, Se(IV), bentonite, and sepiolite did not affect food conversion.

Evaluation of Hg(II) and MeHg Bioaccumulation. To evaluate mercury bioaccumulation, total mercury content of the chickens fed Hg(II) and MeHg supplementation was determined (**Table 4**).

Table 4. Total Mercury Concentrations and Distribution Found in Fresh Weight Chicken Tissues after Hg(II) and MeHg Supplementation

	treatment			
	basal diet + Hg(II) (0.2 mg kg^{-1} in feed)		basal diet + MeHg (0.2 mg kg^{-1} in feed)	
chicken tissue	total Hg[a] (μg kg^{-1})	distribution (%)	total Hg[a] (μg kg^{-1})	distribution (%)
kidney	85 ± 24	15	190 ± 70	2
liver	22 ± 4	12	304 ± 36	10
muscle	6 ± 3	72	113 ± 27	85
skin	1.1 ± 0.5	1	33 ± 9	3

[a] Results of six independent chickens for each group. Three replicates for each measurement (mean value ± SD).

The total Hg contents in the chicken tissues were compared for the two groups to ascertain whether there was any difference in the accumulation process depending on tissue and Hg species exposure. Hg accumulation and distribution pattern were differentially affected by the species ($P > 0.05$), even though the diets had the same total Hg content (**Table 4**).

Total mercury concentrations in the kidney and liver of the chicken treated with Hg(II) were 1 order of magnitude higher than those in muscle or skin. A similar situation was observed for MeHg except for the muscle.

Assuming that 55.6% of carcass weight is muscle, 2.3% is liver, 0.7% is kidney, and 6% is skin and that Hg is evenly distributed throughout these tissues, 11.3 μg of total Hg(II) and 180.4 μg of total MeHg were bioaccumulated in the chickens analyzed. Therefore, this experimental animal study showed a higher MeHg bioavailability (20.7%) compared to Hg(II) bioavailability (1.3%). It was also noted that the greatest body store of Hg(II) and MeHg in chicken was the muscle, kidney being the target organ when Hg(II) was added to the diet and liver when MeHg was added.

Therefore, we can conclude that Hg(II) is not highly bioaccumulated in chicken, kidney being the tissue most sensitive to inorganic mercury poisoning, as previously reported (*6*, *14*, *21*−*23*). This is due to its low gastrointestinal absorption and the fact that inorganic mercury accumulates in the proximal convoluted tubules of the kidney (*24*, *25*), because the urinary route is one of the main pathways for its elimination (*26*). Meanwhile, methylmercury is almost completely absorbed from the gastrointestinal tract (*24*) and excreted to only a very limited extent (*27*), and as a result of MeHg attachment to sulfhydryl groups and binding to proteins on membranes and to enzymes (*26*, *28*), a higher accumulation in the chicken is observed.

All of the control groups showed no evidence of Hg contamination.

Hg(II) and MeHg Bioavailability. *A. Selenium Treatment.* The protective effect of sodium selenite against the toxicity of mercuric chloride, when both compounds are co-administered, in mammals has been known for three decades. The first report on this subject was published by Parizek et al. (*29*), who reported on the alleviation of Hg(II) toxicity by sodium selenite simultaneously administered to rats, showing a protective effect against the renal necrosis and mortality caused by mercuric chloride. Since then, many studies dealing with this phenomenon have been published (*6*, *21*, *23*, *25*, *30*−*35*).

To clarify the interaction between Se and Hg(II) and MeHg and to elucidate its significance, an experimental in vivo study has been carried out. In this particular case we tested Se as a possible antagonist of Hg in broiler chicken.

The Hg levels bioaccumulated in liver, kidney, and muscle of chickens fed with Hg(II) and Se(IV) are shown in **Figure 1**.

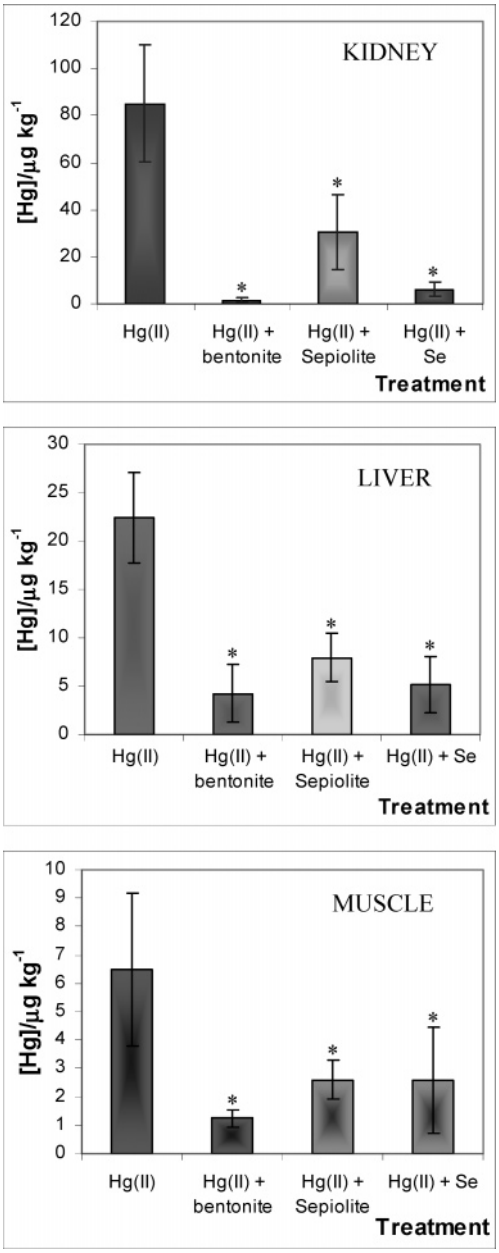

Figure 1. Mercury bioaccumulation in chicken tissues after Hg(II), Hg(II)–bentonite, Hg(II)–sepiolite, and Hg(II)–Se(IV) treatments: *, significant differences between Hg(II)-exposed chickens and [Hg(II) + bentonite]-, [Hg(II) + sepiolite]-, or [Hg(II) + Se]-exposed chickens ($P < 0.05$).

As can be seen, the addition of Se to the Hg(II)-containing diet significantly ($P < 0.05$) alleviated the adverse accumulation of Hg in chicken. The whole-body retention of mercury was drastically decreased, Hg concentration in all tissues being highly affected by Se administration, but the kidney remained the target organ. A drastic reduction in total Hg content between 60 (muscle) and 100% (skin) was observed, the highest concentrations in the kidney being 6 and 85 μg kg^{-1} (wet weight) for chicken with and without Se treatment, respectively.

Hence, not only Hg accumulation but distribution pattern (muscle, 89%; liver, 8%; and kidney 3%) compared to the previous distribution results from **Table 4** was affected by the addition of sodium selenite. This fact is in agreement with other authors who assume that selenium protection must involve a change in the distribution of mercury on a subcellular level (*6*, *21*).

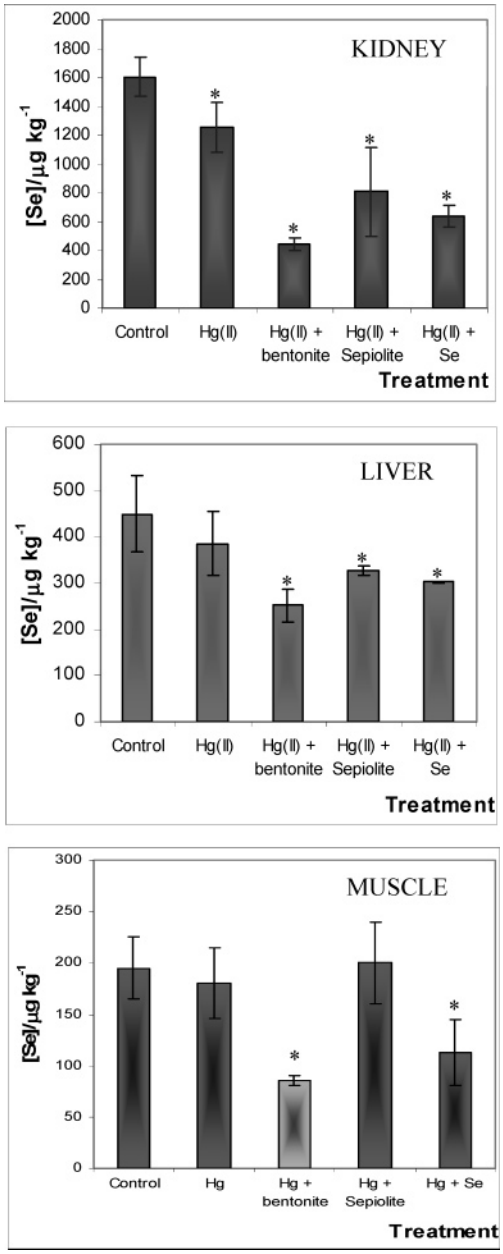

Figure 2. Selenium bioaccumulation in chicken tissues after Hg(II), Hg(II)–bentonite, Hg(II)–sepiolite, and Hg(II)–Se(IV) treatments: *, significant differences between control chickens and [Hg(II)]-, [Hg(II) + bentonite]-, [Hg(II) + sepiolite]-, or [Hg(II) + Se]-exposed chickens ($P < 0.05$).

As a consequence, selenium addition reduced mercury accumulation and promoted a redistribution of Hg from more sensitive organs (kidney, liver) to a less sensitive one (muscle). Therefore, Se influences tissue accumulation through tissue-specific mechanisms.

Several studies on mice, rats, and pigs have shown that Se markedly reduced the mercury content in the kidneys but, in contrast, most of them showed a general trend toward increasing mercury levels in the liver (*21*, *23*, *25*). The addition of selenite to inorganic mercury diets also caused a shift in the tissue mercury distribution.

The presence of Hg(II) did not cause a relevant effect on naturally occurring levels of Se bioaccumulation in liver, muscle, and skin (**Figure 2**). However, it did affect Se accumulation in kidney, but bioaccumulation order (kidney > liver > muscle > skin) remained unaltered.

On the other hand, the addition of Se to the Hg(II)-containing diet affected the Se bioaccumulation in all tissues.

Taking into account that 90% of the total Se has been found to be selenomethionine (SeMet) in the feed used for the target chicken, a possible interaction among SeMet, Hg, and Se(IV) may explain the differences found in Se bioaccumulation under the two different treatments previously described. In fact, the response of animals (rats, chicks) to inorganic mercury after the administration of selenite has been found to be more immediate than that with selenate or selenomethionine (*31*, *33*), but no studies on selenite, selenomethionine, and inorganic mercury presented simultaneously in food have been reported to date.

Furthermore, unlike what happened with Hg accumulation, the Se distribution pattern remained unchanged (muscle, 82−84%; liver, 7−10%; and kidney, 8−9%) in the control group, Hg(II) group, and Hg(II) + Se group. Therefore, mercury inhibited selenium absorption without altering the whole-body distribution of selenium.

Although the mechanism of this finding is not clear, the results are similar to earlier papers showing that dietary exposure to other toxic metal significantly reduces the absorption of selenite in chicks (*31*, *36*, *37*).

The protective effect of sodium selenite used in this study against the toxic accumulation of Hg(II) was as great as had been predicted. Therefore, these improvements should contribute to a solution of a possible inorganic mercury problem in poultry.

In addition, the antagonism by Se of the toxicity of inorganic mercury and its detoxifying effect on MeHg attracted the attention of many scientists in heavy metal toxicology (*32*). Ganther et al. were the first to report the interaction of MeHg and Se. Their results clearly showed the alleviating effects of Se on MeHg-induced mortality and the suppression of weight gain in rats. The results of several studies also suggest that when Se is co-administered with MeHg, the fetotoxicity, neurotoxicity, or developmental toxicity of MeHg is alleviated (*38*). Although many studies on the fate of MeHg in animals have been performed, the mechanisms regulating distribution in tissues and excretion have not yet been examined in detail. Thus, as outlined above, the interactions between Se and MeHg in broiler chickens has been evaluated in this study.

In **Figure 3** the mean values of MeHg expressed as total mercury concentration found in the liver, kidney, and muscle are presented. As can be seen, the addition of Se to the MeHg-containing diet did not affect the mercury accumulation in kidney ($P < 0.05$), but Hg concentration in liver and muscle was highly affected by Se administration (as selenite), with the liver remaining as the target organ. Under selenite administration, organic Hg concentration in muscle reached kidney levels and 2.8-fold higher bioaccumulation was found in the liver.

Despite this, selenium did not significantly change the relative mercury distribution (muscle, 83−84%; liver, 15−16%; and kidney, 1%) in the MeHg and MeHg + Se groups, respectively.

The effect of Se on MeHg accumulation was different from that expected, with an increase in its accumulation especially in liver.

It is known that Se reduces the biliary secretion of MeHg. Urano et al. (*40*) suggested that the decrease in biliary secretion of MeHg induced by Se may result from inhibition of the pathway for secretion of MeHg from liver to bile, rather than the formation of a complex between methylmercury and selenium. Se may specifically inhibit the activity of the canalicular transporter(s) involved in active transport of GSH from liver to bile (*40*). As a result, a slow biliary excretory

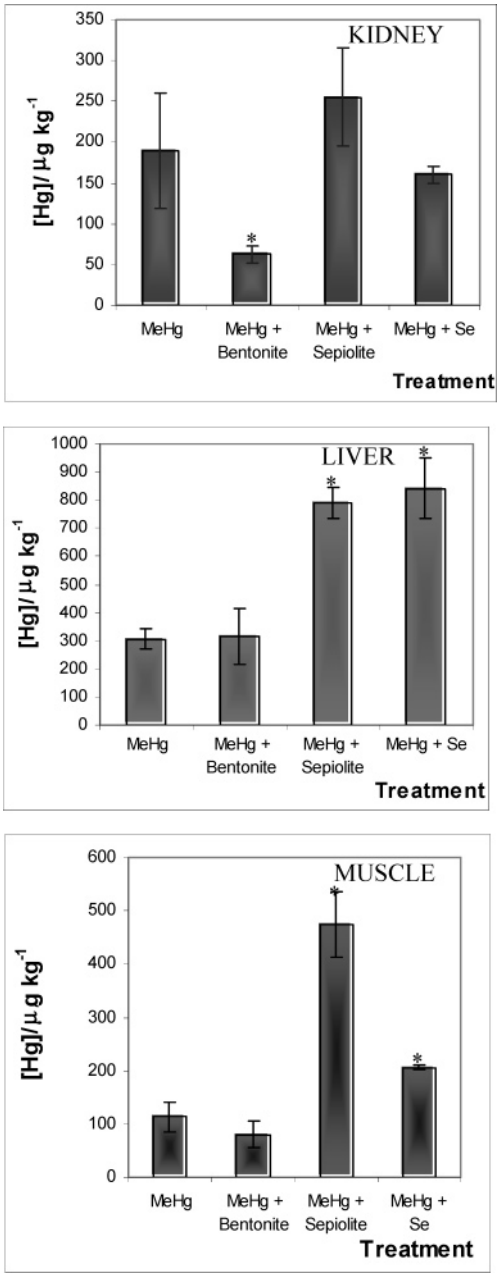

Figure 3. Mercury bioaccumulation in chicken tissues after MeHg, MeHg–bentonite, MeHg–sepiolite, and MeHg–Se(IV) treatments: *, significant differences between MeHg-exposed chickens and [MeHg + bentonite]-, [MeHg + sepiolite]-, or [MeHg + Se]-exposed chickens ($P < 0.05$).

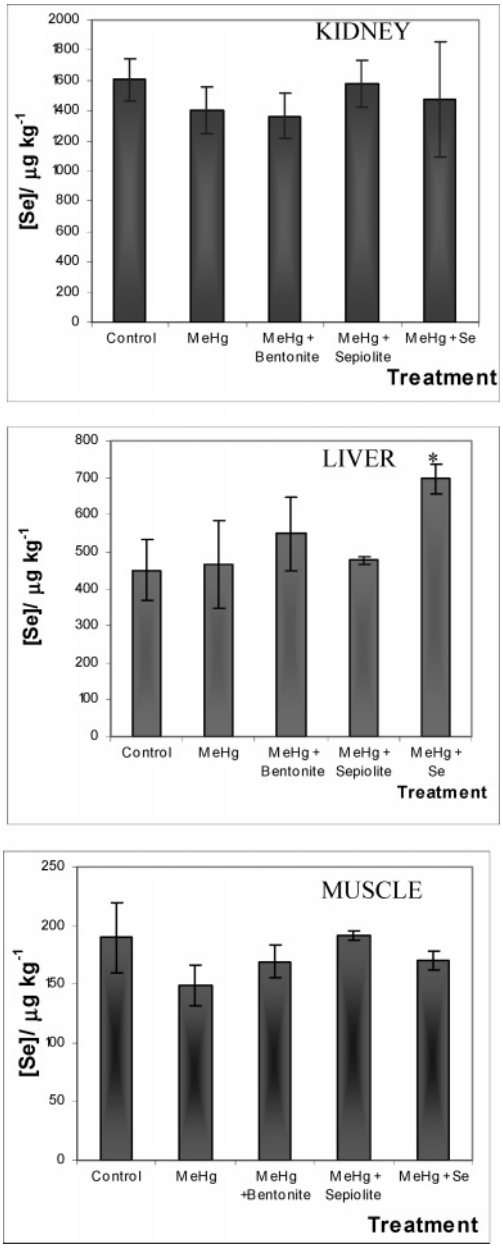

Figure 4. Selenium bioaccumulation in chicken tissues after MeHg, MeHg–bentonite, MeHg–sepiolite, and MeHg–Se(IV) treatments: *, significant differences between control chickens and [MeHg]-, [MeHg + bentonite]-, [MeHg + sepiolite]-, or [MeHg + Se]-exposed chickens ($P < 0.05$).

process would retard whole-body elimination of the metal and favor, thus, its accumulation (*41*).

Therefore, this inhibitory effect of selenium could be due to a MeHg detoxification pathway involving the liver.

Similar results have been previously reported (*40*, *42*−*47*) in which the hepatic Hg level in rats, quails, hens, and mice also increased by the presence of Se.

The presence of MeHg did not cause a relevant effect on naturally occurring levels of Se bioaccumulation (**Figure 4**). In contrast, the addition of Se to the MeHg-containing diet (**Figure 4**) did not affect the Se accumulation in kidney, muscle, and skin, but Se concentration in liver, was increased, with the kidney as the target organ. Furthermore, unlike what happened with inorganic mercury, the Se accumulation pattern was affected when MeHg (muscle, 78%; liver, 11%; and kidney, 10%) or MeHg + Se (muscle, 77%; liver, 14%; and kidney, 9%) was added to chicken feed, in comparison to the control group (muscle, 84%; liver, 7%; and kidney, 9%).

Hence, MeHg promoted Se redistribution from muscle to the liver, suggesting that a mercury−selenium interaction occurred in this organ. This fact also indicates a possible correlation of MeHg and Se, when both are co-administered.

The data presented here emphasize that the relationship between Se and Hg, whereby Hg metabolism by animals is modified, is quite complex and not well understood.

B. Clays Treatment. In this study the evaluation of the capacity to reduce Hg(II) and MeHg accumulation of two different clays (bentonite and sepiolite) was performed.

Results for inorganic mercury are detailed in **Figure 1**. Sepiolite and bentonite incorporation at 2 g kg^{-1} of feed significantly reduces the concentration of Hg in all chicken

tissues [between 64 (muscle) and 100% (skin)], the best results being for bentonite (81−100%).

Results similar to those reported in the present study were found in a study where 5% clipnoptilolite dietary supplement was added to feed enriched with Hg (*14*).

However, the presence of clays in the diet considerably decreases the natural Se content found in the different tissues tested (**Figure 2**), but the bioaccumulation order remained unaltered, kidney > liver > muscle > skin.

Consequently, we concluded that sepiolite and bentonite supplementation may greatly diminish Hg(II) bioaccumulation on broiler chicken. Therefore, these clays could act as promising mercury protectors at low cost.

With regard to MeHg, the results of this experiment are summarized in **Figure 3**. Sepiolite and bentonite have remarkably different behaviors on MeHg bioaccumulation in broiler chickens.

A significant interaction ($P < 0.05$) between bentonite and MeHg is observed in the kidney and muscle, where MeHg expressed as total Hg content was reduced 67 and 29%, respectively, whereas the mercury found in the liver remained unaltered.

On the other hand, the addition of sepiolite to the MeHg-containing diet did not affect Hg accumulation in kidney, but Hg concentration in liver, muscle, and skin was highly affected, the liver being the target organ. Hg concentration in muscle exceeds in this case the levels found in liver without clay addition, leading to 2.6-fold higher bioaccumulation.

As a consequence, sepiolite addition seems to cause an effect on MeHg accumulation similar to that Se addition had on MeHg accumulation. On the other hand, Se bioaccumulation was not reduced (**Figure 4**), in contrast to what happened when clay and Hg(II) were added to the diet.

In conclusion, this study shows that the addition of bentonite to the diet can be beneficial to chickens consuming MeHg-contaminated food. In addition, further studies targeted on Hg are proposed to clarify whether sepiolite enhances MeHg accumulation or MeHg conversion into less toxic forms affects their further accumulation.

ACKNOWLEDGMENT

We thank Almudena Antón and Angel Fernández for their contribution to this work (obtaining, identifying, and collecting of the samples).

LITERATURE CITED

(1) Río-Segade, S.; Bendicho, C. Ultrasound-assisted extraction for mercury speciation by the flow injection-cold vapor technique. *J. Anal. At. Spectrom.* **1998**, *14*, 263−268.

(2) Tao, G.; Willie, S. N.; Sturgeon, R. E. Determination of total mercury in biological tissues by flow injection cold vapor generation atomic absorption spectrometry following tetramethylammonium hydroxide digestion. *Analyst* **1998**, *123*, 1215−1218.

(3) Pyrzynska, K. Analysis of selenium species by capillary electrophoresis. *Talanta* **2001**, *55*, 657−667.

(4) Egeland, G. M.; Middaugh, J. P. Balancing fish consumption benefits with mercury exposure. *Science* **1997**, *278*, 1904−1905.

(5) Ebdon, L.; Pitts, L.; Cornelis, R.; Crews, H.; Donard, O. F. X.; Quevalliller, P. *Trace Element Speciation for Environment and Health*, 1st ed.; Royal Society of Chemistry: Cambridge, U.K., 2001.

(6) Cuvin-Aralar, M. A. A.; Furness, R. W. Mercury and selenium interaction: a review. *Ecotoxicol. Environ. Saf.* **1991**, *21*, 348−364.

(7) Das, K.; Jacob, V.; Bouquegneau, J. M. White-sided dolphin metallothioneins: purification, characterisation and potential role. *Comp. Biochem. Physiol. Part C* **2002**, *131*, 245−251.

(8) Gailer, J.; George, G. N.; Pickering, I. J.; Madden, S.; Prince, R. C.; Yu, E. Y.; Denton, H. S.; Younis, H. S.; Aposhian, H. V. Structural basis of the antagonism between inorganic mercury and selenium in mammals. *Chem. Res. Toxicol.* **2000**, *13*, 1135−1142.

(9) Hoffman, D. J.; Heinz, G. H. Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. *Environ. Toxicol. Chem.* **1998**, *17*, 161−166.

(10) Abad, E.; Llerena, J. J.; Saulo, J.; Caixach, J.; Rivera, J. Comprehensive study on dioxin contents in binder and ant-caking agent feed additives. *Chemosphere* **2002**, *46*, 1417−1421.

(11) Lindberg, A.; Ask Bjornberg, K.; Vahter, M.; Berglund, M. Exposure to methylmercury in non-fish-eating people in Sweden. *Environ. Res.* **2004**, *96*, 28−33.

(12) Savage, G. P. Mercury in fish and fishmeals. Trace Elements: Roles and Risks. *Proceedings of the New Zealand Trace Elements Group Conference*; 1992; pp 11−22.

(13) Ask Bjornberg, K.; Vahter, M.; Petersson-Grawé, K.; Glynn, A.; Cnattingius, S.; Darnerud, P. O.; Atuma, S.; Aune, M.; Becker, W.; Berglund, M. Methylmercury and inorganic mercury in Swedish pregnant women and in cord blood: influence of fish consumption. *Environ. Med.* **2003**, *111*, 637−641.

(14) Lysenco, M. Prevention of mercury accumulation in broiler organs. *Poult. Int.* **2000**, 38−39.

(15) Ouhida, I.; Pérez, J. F.; Piedrafita, J.; Gasa, J. The effects of sepiolite on broiler chicken diets of high, medium and low viscosity. Productive performance and nutritive value. *Anim. Feed Sci. Technol.* **2000**, *85*, 183−194.

(16) Martindale, W. *The Extra Pharmacopoeia*, 28th ed.; Pharmaceutical Press: London, U.K., 1982; p 2025.

(17) Viseras, C.; Lopez-Galindo, A. Pharmaceutical application of some Spanish clays (sepiolite, palygorskite, bentonite): some preformulation studies. *Appl. Clay Sci.* **1999**, *14*, 69−82.

(18) Kececi, T.; Oguz, H.; Kurtoglu, V.; Demet, O. Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *Br. Poult. Sci.* **1998**, *39*, 452−458.

(19) Ouhida, I.; Pérez, J. F.; Gasa, J. Sepiolite (exal) decreases microbial colonization in the gastrointestinal tract of young broilers fed barley−wheat based diets. *Arch. Zootec.* **2000**, *49*, 501−504.

(20) Santurio, J. M.; Mallmann, C. A.; Rosa, A. P.; Appel, G.; Heer, A.; Dageforde, S.; Bottcher, M. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxins. *Br. Poult. Sci.* **1999**, *40*, 115−119.

(21) Potter, S.; Matrone, G. Effect of selenite on the toxicity of dietary methylmercury and mercuric chloride in the rat. *J. Nutr.* **1974**, *104*, 638−647.

(22) Pribilncova, J.; Marettova, E.; Kosucky, J.; Maretta, M. The effect of phenyl mercury on reproductive performance in laying hens. *Acta Vet. Hung.* **1996**, *44*, 377−387.

(23) Hansen, J. C.; Kristensen, P.; Al-Masri, S. N. Mercury/selenium interaction. *Nord. Vet-Med.* **1981**, *33*, 57−64.

(24) Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M. The chemical cycle and bioaccumulation of mercury. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 543−566.

(25) Frisk, P. Expressions of mercury−selenium interaction in vitro. Comprehensive summaries of Uppsala dissertations from the faculty of medicine, Uppsala, Sweden. *Acta Univ. Uppsaliensis* **2001**, *988*.

(26) Clarkson, T. W. The toxicology of mercury. *Crit. Rev. Clin. Lab. Sci.* **1997**, *34*, 369−403.

(27) Rahman, L.; Corns, W. T.; Bryce, D. W.; Stockwell, P. B. Determination of mercury, selenium, bismuth, arsenic and antimony in human hair by microwave digestion atomic fluorescence spectrometry. *Talanta* **2000**, *52*, 833−843.

(28) Graeme, K. A.; Pollack, C. V. Heavy Metal Toxicity, Part I: Arsenic and Mercury. *J. Emerg. Med.* **1998**, *16*, 45−56.
(29) Parizek, J.; Ostadalova, I. The portective effect of small amounts of selenite in sublimate intoxication. *Experientia* **1967**, *23*, 142−143.
(30) Watanabe, C. Modification of mercury toxicity by selenium: practical importance? *Tohoku J. Exp. Med.* **2002**, *196*, 71−77.
(31) Mykkanen, H. M.; Metsaniitty, L. Selenium−mercury interaction during intestinal absorption of ^{75}Se compound in chicks. *J. Nutr*. **1987**, *117*, 1453−1458.
(32) Imura, N.; Naganuma, A. Possible mechanism of detoxifying effect of selenium on the toxicity of mercury compounds. In *Advances in Mercury Toxicology*; Suzuki, T., Ed.; Plenum Press: New York, 1991; pp 275−288.
(33) Parizek, J.; Benes, I.; Ostadalov, I.; Babicky, A.; Benes, J.; Lener, J. Metabolic interrelations of trace elements the effect of some inorganic and organic compouds of selenium on the metabolism of cadmium and mercury in the rat. *Physiol. Bohemoslov.* **1969**, *18*, 95−103.
(34) Kossakowski, S.; Grosicki, A. Interaction between mercury and macro- and microelements in animals. *Pol. J. Environ. Stud.* **1996**, *5*, 5−9.
(35) Grosicki, A.; Kowalski, B. Lead, cadmium and mercury influence on selenium fate in rats. *Bull. Vet. Inst. Pulawy* **2002**, *46*, 337−343.
(36) Mykkanen, H. M.; Humaltoja, T. Effect of lead on the intestinal absorption of sodium selenite and selenomethionine in chicks. *Biol. Trace Elem. Res.* **1984**, *6*, 11−17.
(37) Jensen, L. S. Modification of selenium toxicity in chicks by dietary silver and copper. *J. Nutr.* **1975**, *105*, 769−775.
(38) Naganuma, A.; Ishii, Y.; Imura, N. Effect of administration sequence of mercury chloride and sodium selenite on their fates and toxicities in mice. *Ecotoxicol. Environ. Saf.* **1984**, *8*, 572−580.
(39) Ganther, H.; Goudie, C.; Sunde, M.; Kopecky, M.; Wagner, S.; Hoekstra, W. Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science* **1972**, *175*, 1122−1124.
(40) Urano, T.; Imura, N.; Naganuma, A. Inhibitory effect of selenium on biliary secretion of methylmercury in rats. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 862−867.
(41) Storelli, M. M.; Marcotrigian, G. O. Mercury speciation and relationship between mercury and selenium in liver of *Galeus melastomus* from the Mediterranean Sea. *Bull. Environ. Contam. Toxicol*. **2002**, *69*, 516−522.
(42) El-Begearmi, M. M.; Sunde, M. L.; Ganther, H. E. A mutual protective effect of mercury and selenium in Japanese quail. *Poult. Sci.* **1977**, *56*, 313−322.
(43) Sell, J. L.; Gorani, F. G. Influence of selenium on toxicity and metabolism of methylmercury in chicks and quail. *Nutr. Rep. Int.* **1976**, *14*, 439−447.
(44) Heinz, G. H.; Hoffman, D. J. Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards. *Environ. Toxicol. Chem.* **1998**, *17*, 139−145.
(45) Johnson, S.; Pond, W. G. Inorganic vs organic Hg toxicity in growing rats: Protection by dietary Se but not Zn. *Nutr. Rep. Int.* **1974**, *9*, 135−147.
(46) Emerick, R. J.; Palmer, I. S.; Carlson, C. W.; Nelson, R. A. Mercury−selenium interrelationships in laying hens. *Fed. Prot.* **1976**, *35*, 577.
(47) Nielsen, J. B.; Andersen, O. The toxicokinetics of mercury in mice offspring after maternal exposure to methylmercury—effect of selenomethionine. *Toxicology* **1992**, *74*, 233−241.

Received for review October 19, 2004. Revised manuscript received December 22, 2004. Accepted December 23, 2004. We gratefully acknowledge financial support from FUNDISA. We thank Complutense University for its support through a predoctoral fellowship of A.C.

JF048267V