http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (21): 1373-1377, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1373.1377

Analysis of Heavy Metal Lead (Pb) Levels with Aas in Cow's Milk by Giving Cumin (*Cuminum cyminum* L.), White Turmeric (*Curcuma zedoaria* Rosc.) and Mango Turmeric (*Curcuma mangga* Val.)

[1]E. Nurdin, [2]D.P. Putra and [2]T. Amelia
[1]Faculty of Animal Science, Andalas University, Kampus Unand Limau Manis, Padang 25163, Indonesia
[2]Faculty of Pharmacy, Andalas University, Kampus Unand Limau Manis, Padang 25163, Indonesia

Abstract: The aim of this study was to determine the effect of giving Cumin (*Cuminum cyminum* L.), White Turmeric (*Curcuma zedoaria* Rosc.) and Mango Turmeric (*Curcuma mango* Val.) on levels of heavy metals lead (Pb) in cow's milk produced. The study was conducted in West Java with experimental method in 16 Fries Holland dairy cows with lactation period of 2-4 months and lactation months of 3-4 months. The design used is simple randomized design with 4 treatments such as Group A (control /no treatment), Group B (Cumin 0.03% body weight), Group C (White Turmeric 0.02% body weight) and Group D (Mango Turmeric 0.06% body weight). Measurement of Pb levels in milk using the method of wet destruction, while Pb measurements on faeces using wet ashing method, by means of Atomic Absorption Spectrophotometry. Based on the researsch results showed that administration of Cumin, White Turmeric and Mango Turmeric have very real effect on reducing levels of heavy metals lead (Pb) in cow's milk produced, with a consecutive decrease 98.36, 99.33 and 99.37% and the very real effect on elevated levels of Pb in faeces by 68.01, 64.52 and 80.54%. Mango Turmeric is the best treatment of three treatment in decreasing lead level in milk.

Key words: Milk, Pb, cumin, white turmeric, mango turmeric

INTRODUCTION

Demand for milk is increasing in line with the increasing prosperity of the population. However, the milk produced in several countries, such as Australia, Greece and Hongkong, is contaminated by heavy metals lead (Pb) (Corrigan and Seneviratna, 1990). Meanwhile in Indonesia, the condition is also found and some of them exceed the threshold value and it was also possible that the milk of other places was contaminated (Indraningsih *et al*., 2004; Winugroho and Widiawati, 2009). Nurdin *et al*. (2011a) also obtained data that fresh milk from a dairy farm in West Java had Pb levels varied between 0.56 to 1.31 ppm while the maximum threshold Pb contamination in dairy products amounted to 0.02 ppm (European Commission, 2006; SNI, 2009).

Data released by Bapedal DKI in 1998, the levels of Pb in the big cities' air like Jakarta has reached an average of 0.5 micrograms per cubic meter. For a certain region, such as bus stations and traffic areas, the levels of Pb in the air can reach 2-8 micrograms per cubic meter (Laconi, 2008).

Increased activities that cause environmental pollution, such as transportation and industry, may increase toxic metals level that pollute the environment and contaminate livestock feed and may exceed the maximum threshold. It led to the discovery of the heavy metals in foods or animal products, such as lead (Pb), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni) and zinc (Zn) (Sibuea, 2000).

Pb in the body primarily bound with -SH groups in the protein molecules and inhibit the activity of the enzyme system (Darmono, 2001).Approximately 10-30% inhalated Pb is absorbed by lungs, 5-10% of the ingested is absorbed through the gastrointestinal tract and 30-40% Pb is absorbed through the respiratory tract into the bloodstream. The entry of Pb into the blood stream depends on the particle size, solubility, volume of respiratory and physiology variation between individuals. Pb effects is very harmful to humans, especially to children, such as affect cognitive function, learning ability, shortened stature, hearing impairment, behavior and intelligence, impair the function of organs, such as kidneys, nervous and reproductive system, raise blood

Corresponding Author: E. Nurdin, Faculty of Animal Science, Andalas University, Kampus Unand Limau Manis, Padang 25163, Indonesia

pressure and affect brain development. Pb metals can accumulate in bones, as in the form of Pb^{2+} ions can replace the presence of Ca^{2+} ions in bone tissue (Palar, 1994; Johnson, 1998; Underwood, 2004).

Herbs like cumin (*Cuminum cyminum* L.), white turmeric (*Curcuma zedoaria* Rosc.) and mango turmeric (*Curcuma mangga* Val.) can optimize rumen ecology thus increasing the total rumen bacteria (Nurdin *et al*., 2011a). Increasing number of rumen bacteria will increase the amount of heavy metals (Pb) which is excreted through faeces (Sunaryadi, 2006). The content contained on the cumin, white turmeric and mango turmeric such as flavonoids, phenolic and saponin as antioxidants and anti-inflammatory can increase the permeability of the cells and then the immune system will increase (Nurdin and Susanti, 2009). Administration of 0.03% cumin, 0.02% white turmeric and 0.06% mango turmeric in dairy cattle feed is the best dose to improve rumen ecological conditions become optimum, improving livestock productivity and increase endurance (Nurdin and Arief, 2009; Nurdin *et al*., 2011b). Possibly content of phenols in cumin, white turmeric and mango turmeric will play an important role in decreasing levels of heavy metals Pb.

Based on the description, research was conducted to determine the effects of cumin, white turmeric and mango turmeric in dairy cows feed to the levels of heavy metals Pb in milk yield.

Analysis of Pb content in milk performed by wet destruction method, whereas Pb analysis in faeces is done by wet incineration. Furthermore, Pb levels is measured by Atomic Absorption Spectrophotometry (AAS) instrument. AAS working principle is based on the evaporation of the sample solution and then the metal contained in solution is converted into free atoms. The atoms absorb the radiation emitted from the light source of the lamp cathode (Hollow Cathode Lamp) containing elements to be determined. Then amount of radiation absorption measured at specific wavelengths by type of metal (Darmono, 1995).

MATERIALS AND METHODS

This study was done in smallholder dairy farm in Lembang, West Java for three months. Pb levels on milk and faeces was analyzed by Atomic Absorption Spectrophotometry AA-100 and collected data were analyzed by analysis of variance. After a significant F test ($p<0.05$), Duncan's Multiple Range Test was used to inspect differences among treatment (Darmono, 1995).

Herbs preparation: The materials used (cumin, white turmeric, mango turmeric) prepared in a fresh condition. Materials are washed, drained and chopped with a thickness of +0.1 cm, then aerated in open space protected from direct sunlight for 1 week. Materials that have been dried are pounded to obtain the materials in powder form.

Preparation of experimental animals: Sixteen Fries Holland dairy cattle were used in this experiment. Experimental animals were randomly selected and in 2nd-4th lactation period and 3rd-4th lactating months with average body weight of 450 kg. Formerly cows were given Benvet GR 250 which contains 250 mg of albendazole.

Treatment of experimental animal: A simple randomized design is used with four treatments and four replications. Prior to treatment, Benvet GR was given. After 14 days, initial sample of environment (water used on the farm, forage, concentrates and tofu waste) cow's milk and faeces was taken. The next day, the dairy cows were given 6 kg tofu waste, 6 kg of concentrate and 40 kg of hijauan. Then in cattle feed was given the treatment:

- Group A : Control (no herbal giving)
- Group B : Cumin (0.03% of body weight)
- Group C : White turmeric (0.02% of body weight)
- Group D : Mango turmeric (0.06% of body weight)

The treatment is given once a week for two months of the study.

Initial sampling: Initial samples taken from dairy cattle milk that has been designated as samples and samples from the environment, such as water, forage, concentrates, tofu waste and cow faeces. The initial sample was taken two weeks after the administration and the day before dairy cattle treated by Benvet GR.

Final sampling: The final sample taken from the evening milk yield and faeces after the treatment ended. Cow's milk is taken as 200 mL and cow faeces as much as 100 g of each cow and milk samples were stored in a refrigerator for analysis in the next day.

Preparation of Pb standard solution:

- Preparation of Pb master raw solution 1000 $\mu g\ mL^{-1}$
- $Pb(NO_3)_2$ were weighed as much as 0.4 g. Put in a 250 mL flask, then dissolved with HNO_3 0.15 N to boundaries mark
- Preparation of Pb Standard Solution Pipette 10 mL of Pb raw solution, put in 100 mL flask, align to boundaries mark with HNO_3 0.15 N. Pipette much as 1, 2, 3, 4, 5 and 6 mL into a 50 mL flask, align to boundaries mark with HNO_3 0.15 N, whipped 12 times. Retrieved lead standard solution 2 mg mL^{-1}; 4, 6, 8, 10 and 12 $\mu g\ mL^{-1}$

Pb absorption measurement of initial and final sample: For cow's milk and water, was done by wet destruction. Put 5 g sample in erlenmeyer 125 mL, add 10 mL of concentrated HNO_3. Then heated on a hot plate with a temperature of 110°C for 1 h. Then remove and chill it, filtered with Whatman paper No. 41, put in 50 mL measuring flask, rinse with ion-free water and dilute to the mark. Filtrate was ready for the analysis (Darmono, 1995).

For forage, concentrates, tofu waste and cow faeces, done by wet ashing. Weigh 1 g of fresh sample in a measuring cup, then add 7 mL HNO_3 to sample sink. Then heated at a low temperature (80°C) for 4 h until yellowish. Aerate for 1 night without heating. The next day, add 2 mL of sulfuric acid and heat at 120°C for 1 h. Add/drops 0.1 to 0.6 mL or 4-6 drops of solution (a mixture of 2 parts of perchoric acid and 1 part nitric acid, heat for 10-15 min. Add 2 mL distilled water and 0.6 mL of hydrochloric acid, heat 120°C for 15 min, then chill and dilute to 50 mL and shake. Take 10 mL, read using AAS (Reitz *et al*., 1960).

Pb levels calculation:

$$\text{Lead levels (ppm)} = \frac{X \cdot Y}{Z}$$

where, X is the concentration obtained by the calibration curve (μg mL^{-1}), Y is the volume of sample solution (mL) and Z is the weight of the sample (g).

RESULTS AND DISCUSSION

The results of absorbance measurements series of standard solutions of lead (II) nitrate to made calibration curve at a wavelength of 283.3 nm using a Pb hollow cathode lamp produced regression equation y = 0.0255x-0.0013 with a correlation coefficient (r) = 0.99925.

The results of determination of lead contamination levels in the environment is water by 28.81 ppm, forage by 26.47 ppm, concentrates by 0:08 ppm and tofu waste by 1.2 ppm. The high Pb contamination probably caused by the location of the research is in the area Tangkuban Perahu volcano, so it naturally became a contributor to high levels of Pb in the environment, because Pb is naturally derived from the geological weathering and volcanic eruptions (Manahan, 1992). Moreover, the place is an attractive area that many vehicles passing by. This area is also densely populated and industrial areas of medicine, tofu and textiles that contribute substantial Pb on the environment.

The results of lead metal contamination determination at the initial examination was 1.1514 ppm for faeces and 5.3484 ppm for milk.

The result of the levels of lead metal contamination in the milk after a given treatment: group A of 6.8998+2073 ppm, group B of 0.1097+0.0585 ppm, the C of 0.0338+0.0390 ppm and group D of 0.0093+0.0062 ppm and in faeces: group A of 0.9635+0.1205 ppm, group B of 3.7196+0.1342 ppm, group C of 3.1288+0.3955 ppm and group D of 4.7645+0.442 ppm.

Table 1: Treatments effect on Pb levels in milk (ppm)

	Treatments			
	A	B	C	D
1	9.6690	0.0512	0.0000	0.0200
2	7.3425	0.2510	0.0000	0.0173
3	8.8763	0.0227	0.1350	0.0000
4	1.7113	0.1140	0.0000	0.0000
Total	27.5991	0.4389	0.1350	0.0373
Average	6.8998±2.073^{a}	0.1097±0.0585^{b}	0.0338±0.0390^{b}	0.0093±0.0062^{b}

Different superscripts indicate highly significant different effects ($p<0.01$) A: Control, B: Cumin (0.03% weight), C: White turmeric (0.02% weight), D: Mango turmeric (0.06% of body weight)

Table 1 shows that the highest Pb content contained in a treatment without given herbal treatment, while the lowest Pb content contained on treatment D which is treated by administering mango turmeric as much as 0.06% of body weight. This suggests that administration of cumin can reduce Pb levels of milk by 98.36%, white turmeric by 99.33% and mango turmeric by 99.37%.

Based on the T test results of four treatment group, there were no significant difference between before and after treatment in group A (control). While in group B, C and D, highly significant differences were found between the levels of Pb before and after treatment, so it can be concluded that administration of cumin, white turmeric and mango turmeric have an influence on the decrease in Pb levels in milk.

Based on the one way varian analysis results, it was known that the administration of cumin, white turmeric and mango turmeric has a very significant difference to the levels of Pb in milk compared with treatment without given herb. After Duncan tests, group B, C and D showed highly significant differences with group A, but did not show significant differences between treatments B, C and D that are treated by administering cumin, turmeric, white turmeric and mango. It can be concluded that administration of cumin, white turmeric and mango turmeric showed the same ability to decrease levels of Pb in milk.

In group A, Pb levels at the end in milk increased by 9.35% of the initial levels. It was due to feed dairy cows in this study site as forage, tofu waste, water and air has a high level of Pb contamination. So the cattle will consume these contaminated materials every day. This is accordance with the opinion by Gravert (1987) which states that the level of contamination in milk is depend on the levels of contaminants that taken with food by the cattle. Contamination in feed, water and air will be released into the milk as much as 5-10% of the amount of contaminants consumed.

Table 2: Treatment effect on Pb levels in faeces (ppm)

	Treatments			
	A	B	C	D
1	0.7110	3.8970	3.4900	5.0770
2	0.9480	3.3918	3.2120	4.1221
3	1.2220	3.7177	2.1411	4.1670
4	0.9730	3.8717	3.6720	5.7017
Total	3.8540	14.8782	12.5151	19.0578
Average	1.3785 ± 0.1205^{A}	3.7196 ± 0.1342^{B}	3.1288 ± 0.3955^{b}	4.7645 ± 0.442^{C}

Different superscripts indicate highly significant different effects ($p<0.01$) A: Control, B: Cumin (0.03% weight), C: White turmeric (0.02% weight), D: Mango turmeric (0.06% of body weight)

Table 3: Phytochemical screening result of cumin, white turmeric and mango turmeric

Contents	Cumin	White turmeric	Mango turmeric
Flavonoid	+	+	+
Saponin	+	+	+
Triterpenoid	-	+	+
Steroid	-	+	+
Kuinon	-	-	+

Source: Nurdin and Susanti (2009)

The content of phenolic compounds in cumin, white turmeric and mango turmeric role in reducing the levels of Pb in cow's milk. Turmeric mango has the highest activity in reducing Pb levels in cow's milk. Pb decreased in cow's milk because Pb easily bound together by fatty acids bonds which generally become constituent of natural antioxidants in herbs Nurdin *et al*. (2011b). Pb-phenolic compounds bound form a molecule with large BM and difficult to absorbed by the body and excreted through faeces. The more Pb bound, the more Pb excreted through faeces, as seen in Table 2.

Table 2 shows that the highest Pb content contained in treatment D, treatment with mango turmeric (0.06% body weight). Followed by treatment B which is treated with cumin (0.03% body weight), treatment C which is treated with white turmeric (0.02% body weight). The lowest Pb content contained in treatment A, treatment without giving herb. This was suggested that administration of cumin can increase Pb levels of faeces by 68.01%, white turmeric by 64.52% and mango turmeric by 80.54%. Pb level of faeces decreased in group A. After the t-test, it was known that decreased levels of Pb in faeces of group A after treatment did not have significant differences with Pb levels in the faeces before treatment.

Decreasing levels of Pb in milk inversely related to elevated levels of Pb in faeces. However, the average decline of Pb levels in cow's milk by 99.02% are not comparable with the average amount of Pb excreted through the faeces of 71.02%. This is compatible with the stated by Palar (1994) that the Pb metal not only excreted via faeces, but also through urine and sweat, so elevated levels of Pb in the faeces is not comparable with decreased levels of Pb in cow's milk.

Based on the results of phytochemical test, cumin, white turmeric and mango turmeric contains polyphenols, flavonoids, quinones and saponin compounds as antioxidants, anti-inflammatory and antibacterial that can be seen in Table 3 (Nurdin and Susanti, 2009).

Saponin content of cumin, white turmeric and mango turmeric can reduce the population of protozoa in the rumen (Bunda, 2010) so it can increase the amount of rumen bacteria. The increasing number of rumen bacteria can increase the amount of heavy metals which are excreted through the faeces. This is compatible with those expressed by Sunaryadi (2006) that the higher the number of rumen bacteria, the greater the levels of heavy metals in milk decreased and increase the levels of heavy metals in the faeces.

In addition, administration of cumin in dairy cattle may decrease NH_3 concentration of rumen fluid. The greater the number of rumen bacteria, the smaller the NH_3 concentration, because the bacteria utilize NH_3 as a source of nitrogen for growth and breeding (Satter and Slyter, 1974; Nurdin and Arief, 2009; Nurdin *et al*., 2011b). Cumin can also make optimum ecological conditions in the rumen at pH 6-7, where the rumen microbes can grow well (Church, 1996; Nurdin and Arief, 2009). This is compatible with the results obtained that the administration of cumin, white turmeric and mango turmeric can raise the levels of Pb excreted in the faeces and Pb accumulated in the milk reduced (Table 1 and 2).

CONCLUSION

Supplementation of Cumin, White Turmeric and Mango Turmeric significantly reduced Pb levels of milk and increased Pb levels that excreted through faeces. The giving of Cumin decreased Pb levels in milk by 98.36%, White Turmeric 99.33% and Mango Tumenric 99.37% and elevated levels of Pb in faeces by 68.01%, White Turmeric 64.52% and Mango Turmeric 80.54%.

ACKNOWLEDGMENT

This experiment was funded by Directorate General of Higher Education, Ministry of National Education, through "Hibah Kompetitif Penelitian Strategis Nasional"No.006/SP2H/PL/Dit.Litabmas/III/2012.

REFERENCES

Bunda, D.O.S., 2010. Effect of gambir leaf condition on microbial population and characteristics of rumen fluid by *in-vitro*. Ph.D. Thesis, Universitas Andalas, Padang, Indonesia.

Church, D.C., 1996. Digestive Physiology and Nutritional of Ruminant. Prentice Hall, New Jersey.

Corrigan, P.J. and P. Seneviratna, 1990. Occurrence of organochlorine residues in Australian meat. Aust. Vet. J., 67: 56-58.

Darmono, 1995. Metals in Biological System of Organism. University of Indonesia Press, Jakarta, Indonesia.

Darmono, 2001. Environment and Pollution. University of Indonesia Press, Jakarta, Indonesia.

European Commission, 2006. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official J. Eur. Union, L364: 5-24.

Gravert, H.O., 1987. Dairy Cattle Production. Elsevier Science Publisher Inc., Amsterdam, Netherlands.

Indraningsih, Y. Sani, R. Widiastuti, E. Masbulan and G.A. Bonwick, 2004. Minimalization of pesticide residues in animal products. Prosiding Seminar Nasional Parasitologi dan Toksikologi Veteriner, (2004), Balai Penelitian Veteriner dan Department for International Development, UK., pp: 105-126.

Johnson, F.M., 1998. The Genetic effect of environmental lead. Mut. Res., 410: 123-140.

Laconi, E.B., 2008. Dangers of lead heavy metal contamination in food. http://www.hydro.co.id /2011/06/23/bahaya-kontaminasi-logam-berat-timbal-pada-makanan/.

Manahan, S.E., 1992. Toxicological Chemistry. Lewis Publisher, New York.

Nurdin, E. and A. Arief, 2009. The effectivity of cumin as natural anti-oxidant to improve rumen ecology of mastitis dairy cow's. Anim. Prod., 11: 160-164.

Nurdin, E. and H. Susanti, 2009. Utilization of natural antioxidants on mastitis dairy cattle productivity. Research Report of Competitive Grant Research Report Corresponding National Priority Batch III. Directorate General of Higher Education. Ministry of Education, Indonesia.

Nurdin, E., F. Susanti, T. Amelia and U.H. Tanuwiria, 2011a. Utilization of herbals and Cu-Zn propionate on Pb heavy metal contamination *in-vitro*. Proceedings of National Seminar Animal Husbandry and Veterinary Technology Center for Research and Development of Animal Husbandry, (2011), Bogor, West Java, Indonesia, pp: 39-40.

Nurdin, E., T. Amelia and M. Makin, 2011b. The effect of herbs on milk yield and milk quality of mastitis dairy cows. J. Indonesian Trop. Anim. Agric., 36: 104-108.

Palar, H., 1994. Contamination and Metals Toxicology. Rineka Cipta Publsher, Jakarta, Indonesia.

Reitz, L.L., W.H. Smith and M.P. Plumlee, 1960. Simple, wet oxidation procedure for biological materials. Anal. Chem., 32: 1728-1728.

SNI, 2009. Maximum limits of heavy metal contamination in food. Agency of Standarization National, SNI. No.7387:2009.

Satter, L.D. and L.L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production *In vitro*. Br. J. Nutr., 32: 199-208.

Sibuea, P., 2000. Dangers of lead heavy metal contaminationn in food. Mater of Science in Food Technology. University of Gadjah Mada, Yogyakarta, Indonesia. http://www.sedapsekejap. com/.

Sunaryadi, 2006. Soaking toxicity of lead (Pb) with organic mineral supplementation, chitosan and brown seaweed extract. Ph.D. Thesis, Institut Pertanian Bogor. Bogor, Indonesia.

Underwood, J.C.E., 2004. General and Systemic Pathology. 4th Edn., Churchill Livingstone, Edinburgh, Scotland.

Winugroho, M. and Y. Widiawati, 2009. Effect of bioplus probiotics on heavy metal content of Pb in cow's milk in Northern bandung territory (Lembang). Research Report on APBN Research. BPPT. Bogor, Indonesia.