Accumulation and Degradation of Thiabendazole Residues in Eggs in Administered Layer Hens

Mahadeva Naika¹* and Farhath Khanum²

¹Applied Nutrition Division, Defence Food Research Laboratory, Siddarthanagar, Mysore-570011, India
²Biochemistry and Nano Sciences, Defence Food Research Laboratory, Siddarthanagar, Mysore-570011, India

Abstract

Administration of thiabendazole (TBZ) to laying hens may cause accumulation of the drug residues in eggs. Twenty five week old hens (n=6) were administered with a single oral dose of 75 mg TBZ/day/layer hen for 5 consecutive days and its residues in eggs were quantified by HPLC-FD. The highest TBZ (0.4308±0.0253 ppm), below maximum residue limit (0.0886±0.0161 ppm) and below limit of detection (LOD) residue concentrations in eggs were observed on mean time of 127.54, 177.62 and 252.58 h after first feeding, respectively. The highest TBZ concentration (0.5260 ppm) and total residue on any day from 2 to 9 day in the entire egg white portion were significantly (P<0.01) higher than those in the yolk. The highest residue concentrations as well as the highest total residue in egg albumin (0.5944 ppm), yolk (0.6587 ppm) and whole egg (0.5867 ppm) were quantified. The highest TBZ concentration (0.5260 ppm) as well as total TBZ residue on any day from day 2 to day 9 in the entire white portion of egg were significantly (P<0.01) higher than those in the yolk. Egg white, yolk and whole egg from the TBZ fed hens had residues at 50.71 to 153.50, 75.62 to 153.50 and 50.71 to 153.50 h after first feeding, respectively. The highest concentration TBZ residue in white egg is 0.4308±0.0253 ppm and cumulative residue is 8.1886±6.5385 ppm and also calculated in microgram is useful to show as per the standard values. The work indicated that safe dose and days to consume egg. Safe time/days can be reduced for consuming eggs after administration of thiabendazole by using different types of decontamination techniques.

INTRODUCTION

Use of several veterinary drugs on food-producing animals, grown intensively by modern animal husbandry practices, is common to many countries (Mellon et al., 2001; Solomon et al., 2002; Wachtel et al., 2002; Goldman 2004 and Cam et al., 2009). Thiabendazole (TBZ), registered in many countries, is used as a broad-spectrum anthelmintic agent to control parasitic infestations in animals. It is also used as antifungal as well as growth promoting agent on animals. Inappropriate use of veterinary drugs on animals as well as in feeds and foodstuffs has led to their increased residues in human food commodities like eggs in many countries including India (Schaellibaum 1990; Lidong 1992; Paige 1994; Reja-Sanche et al., 1995 and Okerman et al., 2001).

As a direct hazard, chronic and acute toxicities such as teratogenesis, carcinogenicity, liver hypertrophy, thyroid hyperplasia, immuno-suppression and decreased foetal as well as maternal body weight have been associated with varying exposures to benzimidazoles including TBZ as a major compound. The most concern for indirect hazard from the use of antibiotics in animal husbandry is the development of drug-resistant pathogens in food animals (Kulshrestha 1990; Brady et al., 1993; Bordas et al., 1997; Gogus et al., 2000 and Wrigley et al., 2006), which in turn, may lead to antibiotic-resistant pathogen in animal-derived foodstuffs and human beings (Willis et al., 1999; Rajashekara et al., 2000; Swartz, 2002; Schlegelova, 2002 and Horby et al., 2003).

Distribution of veterinary drug residues and their dissipation from various parts of animal's body, depending upon type of drugs, animal, organ, meat portion, milk, egg etc., may give rise to their detectable residue levels (Takahashi et al., 1991; Donoghue et al., 1999; Kuehn et al., 2000; Cornelis et al., 2000 and Roudaut et al., 2000). Glomerular filtration and tubular secretion are the major routes of administration of TBZ, faecal elimination through enterohepatic recycling and bone sequestration are the secondary routes for the elimination of administered TBZ from animal body (Bai et al., 2010). Conversely, oral administration of TBZ to sheep, cattle, goats, dogs and humans results rapid absorption from the GI tract, and almost all the entire quantity is recovered from urine and faeces. The hydroxylation of the benzimidazole ring at the 5-position to form 5-OH TBZ and subsequent conjugation to form the glucuronide and sulfate are the major metabolic steps. In a study an oral administration of 3.19
mg of [14C] TBZ daily to laying hens for 10 consecutive days showed an average recovery of 96.6% of the total administered dose, and about 99.6% of this recovered dose was found in the excreta, in the form of both unconjugated (3.4 mg/kg) and conjugated (4.4 mg/kg) 5-OH TBZ (Halls et al., 1991a; Chukwudebe et al., 1994 and Bai et al., 2010). Cumulatively, the total residues found in the tissues and eggs accounted for about 0.4% or less of the 14C. The total residues in eggs attained a level of about 0.1 mg/kg by day 2 and remained relatively unchanged throughout the next 8 days. The residues in tissues and eggs consisted mainly of unconjugated 5-OH TBZ, unmetabolized TBZ and benzimidazole at maximum concentrations, in the kidneys, of 0.4, 0.11 and 0.12 mg/kg respectively. In an animal transfer study, chickens (males and females) treated continuously for 7 weeks with TBZ at levels corresponding to 2, 20, 2000 and 2000 ppm in the feed (Yang et al., 2011), showed the sum of TBZ and 5-OH TBZ including its conjugate as 20-28 ppb in fat taken from different body parts, 17-23 ppb in a 1:1 mixture of breast and leg meat, and 60-80 ppb in liver at the 20 mg/kg feed level. Neither TBZ nor its related residues are likely to persist in milk, eggs or edible tissues because of their relatively low concentrations and rapid elimination. Present study was undertaken to find out the accumulation and degradation levels of TBZ in layer chicken (BV 300) administered with a dose of 75 mg per bird per day for 5 consecutive days.

MATERIALS AND METHODS

HPLC grade water filtered through 0.2 µm (Qualigens Fine Chemicals, Glaxo-SmithKline Pharmaceuticals Ltd), HPLC grade acetonitrile and methanol (Ranbaxy Fine Chemicals Ltd, SAS Nagar, India), filtered through a 0.2 µm membrane) purchased from Sigma-Aldrich Corporation were used. Mobile phase was prepared and filtered through 0.22 µm Millipore Durapore solvent filters (disc, 47 mm, 9.6 cm2 filtration areas) under vacuum with Millipore All-glass Filter unit, degassed. Analytical standard thiabendazole (Sigma-Aldrich) was dissolved in HPLC grade methanol to get 1000 ppm neat stock solution/suspension of 25000 ppm was procured from Sigma-Aldrich Inc, St. Louis, USA. TBZ solution/suspension of 25000 ppm was prepared in distilled water, 3 ml of which was used to administer each of 6 experimental birds with a single oral dose of 75 mg TBZ per bird per day for 5 consecutive days.

RESULTS AND DISCUSSION

Accumulation of TBZ in albumin and yolk of eggs obtained from administered layer hens from after first day of TBZ feeding to till non-detectable days (11th day) and from sacrificed hens at the end of experiment were presented in the form of both individual concentration as well as total residue levels. Subsequently quantified the TBZ levels in eggs on different times (hours) after the administration were shown table 2.

TBZ residue concentration (ppm) as well as total TBZ residue in entire portion of white, yolk or whole egg (white + yolk) with respect to the mean time after first TBZ administration were shown in table 2. TBZ residue was not detected in egg white and yolk on day 1 and day 11 after a mean time of 1.96 and 252.58 h from first feeding, respectively, and non-detectable residue levels were considered as nil residues for statistical analysis and interpretation. In case of egg white, TBZ residues concentration of 0.0888 ppm (i.e., 2.603 μg total residue in the entire white portion) appeared on day 2 (after mean time of 25.87 h from first feeding), reached a maximum concentration level of 0.5260 ppm (i.e., 15.5925 μg total residue) on day 6 (after 127.54 h from first feeding) and declined thereafter to 0.0108 ppm concentration level (i.e., 0.2996 ug total residue) on day 10 (after 227.62 h from first feeding) before reaching non-detectable level on day 11. Similarly for egg yolk, TBZ residues concentration of 0.0215 ppm (i.e., 0.2964 ug total residue in the entire yolk portion) appeared on day 2 (after mean time of 25.87 h from first feeding) and reached maximum concentration level of 0.2157 ppm (i.e., 0.2996 ug total residue) on day 6 (after 127.54 h from first feeding) and declined thereafter to 0.0144 ppm concentration level (i.e., 0.1899 μg total residue) on day 10 (after 227.62 h from first feeding).
Table 2: TBZ residue in eggs and their components (n=6)

| Day and Time of Laying from First Feeding | TBZ Concentration (TC), ppm | TBZ Concentration Ratio (TCR) White/ Yolk (TCW/TCY) |
|------------------------------------------|-----------------------------|---------------------------------------------|
| Day 1, 1.96±1.17 h                       | 0.0±0.0 A                   | -                                           |
| Day 2, 25.87±0.66 h                      | 0.088±0.0176 FG             | 0.0215±0.0064 CD                           |
| Day 3, 50.71±1.16 h                      | 0.3197±0.0380 LM            | 0.0541±0.0139 EF                           |
| Day 4, 75.62±0.93 h                      | 0.3859±0.0438 MN            | 0.1493±0.0188 GH                           |
| Day 5, 101.67±1.02 h                     | 0.4801±0.0370 OP            | 0.2314±0.0254 K                           |
| Day 6, 127.54±1.62 h                     | 0.5260±0.0360 OP            | 0.2314±0.0254 K                           |
| Day 7, 153.50±2.35 h                     | 0.5185±0.0328 PK            | 0.1493±0.0188 GH                           |
| Day 8, 177.62±3.35 h                     | 0.0965±0.0168 FG            | 0.0541±0.0139 EF                           |
| Day 9, 201.37±4.89 h                     | 0.0157±0.0051 BC            | 0.0541±0.0139 EF                           |
| Day 10, 227.62±3.90 h                    | 0.0108±0.0024 B             | 0.0541±0.0139 EF                           |
| Day 11, 252.59±4.58 h                    | 0.0±0.0 A                   | 0.0±0.0 A                                  |

Mean±SD (n=6) values of TBZ total residue in the whole egg and their components (n=6) vary significantly (P<0.01) of 1, 2, 3,... varying significantly (P<0.05). Mean±SD (n=6) values of TBZ residue concentration in the yolk (TCY) vary significantly (P<0.01). Grand mean±SD (N=6X9) values of TBZ concentrations in a particular row, carrying different superscripts I, II, III,... vary significantly (P<0.05).

Table 3: TBZ residue in eggs and their components

| Day and Time of Laying from First Feeding | TBZ Residue Concentration, ppm | TBZ Total Residue, µg |
|------------------------------------------|---------------------------------|-----------------------|
| Day 1, 1.96±1.17 h                       | 0.0±0.0 A                       | 0.0±0.0 A             |
| Day 2, 25.87±0.66 h                      | 0.088±0.0176 FG                 | 0.0215±0.0064 CD     |
| Day 3, 50.71±1.16 h                      | 0.3197±0.0380 LM                | 0.0541±0.0139 EF     |
| Day 4, 75.62±0.93 h                      | 0.3859±0.0438 MN                | 0.1493±0.0188 GH     |
| Day 5, 101.67±1.02 h                     | 0.4801±0.0370 OP                | 0.2314±0.0254 K      |
| Day 6, 127.54±1.62 h                     | 0.5260±0.0360 OP                | 0.2314±0.0254 K      |
| Day 7, 153.50±2.35 h                     | 0.5185±0.0328 PK                | 0.1493±0.0188 GH     |
| Day 8, 177.62±3.35 h                     | 0.0965±0.0168 FG                | 0.0541±0.0139 EF     |
| Day 9, 201.37±4.89 h                     | 0.0157±0.0051 BC                | 0.0541±0.0139 EF     |
| Day 10, 227.62±3.90 h                    | 0.0108±0.0024 B                 | 0.0541±0.0139 EF     |
| Day 11, 252.59±4.58 h                    | 0.0±0.0 A                       | 0.0±0.0 A             |

Mean±SD (n=6) values of TBZ total residue (TC) varying significantly (P<0.01). Mean±SD (n=6) values of TBZ total residue (TC) varying significantly (P<0.01). Cumulative residue (from hens on from day 2 to day 11), mean±SD (N=6X9) values of TBZ total residue in a particular row, carrying different superscripts I, II, III,... vary significantly (P<0.01).
Table 4: TBZ residue in eggs and their components

| TBZ Residue | White   | Yolk    | Whole Egg |
|-------------|---------|---------|-----------|
| Highest residue concentration (ppm), n=6 | 0.5260±0.0360<sup>cd</sup> | 0.2157±0.0290<sup>ab</sup> | 0.7308±0.0253<sup>cd</sup> |
| Highest total residue (µg), n=6 | 15.5925±0.8510<sup>d</sup> | 2.8544±0.4573<sup>a</sup> | 18.4470±0.9598<sup>d</sup> |
| Cumulative residue Mean±SD (µg), n = 60 | 6.9052±5.7154<sup>B</sup> | 1.2834±0.9659<sup>a</sup> | 8.1886±6.5385<sup>B</sup> |
| Cumulative residue of 54 eggs (TBZ) (µg) | 372.88 | 69.30 | 442.18 |
| Cumulative residue of 54 eggs (TBZ) (as % of drug fed) | 0.0994% | 0.0185% | 0.1179% |

1% a, b, c, …; 5% 1, 2, 3, …; and 2% A, B, C, …; TBZ

CONCLUSIONS

No significant effect due to TBZ administration was observed on birds’ live weight, laying time and egg weight during the experiment. Upon TBZ administration, the highest residue (0.4308±0.0253 ppm), the < MRL (0.1 ppm) residue (0.0886±0.0161 ppm) as well as the < LOD residue concentrations in eggs were observed in the mean time of 127.54, 177.62 and 252.58 h after first feeding, respectively. The highest TBZ concentration (0.5260 ppm) as well as total TBZ residue on any day from day 2 to day 9 in the entire white portion were significantly (P<0.01) higher than those in the yolk (table 2 and 3). Egg white, yolk and whole egg from the TBZ treated hens had violation residues on 50.71 to 153.50, 75.62 to 153.50 and 50.71 to 153.50 h after first feeding, respectively. The highest concentration of TBZ residue in whole egg was observed 0.4308±0.0253ppm and the cumulative residue 6.1886±6.5385ppm, and also calculated in microgram is useful to show as per the standard values (table 4).

ACKNOWLEDGEMENTS

We are thankful to Dr. H.V Batra, Director, Defence Food Research Laboratory Mysore for kind permission to publish this paper.

REFERENCES

Arenas, R.V. and Johnson, N.A. (1995). Liquid Chromatographic Fluorescence Method for Multiresidue Determination of Thiabendazole and 5-Hydroxy thiabendazole in Milk. Journal of Association of Official Analytical Chemistry International 78(3): 642-646.

Bai, X., Bai, F., Zhang, K., Lv, X., Qin, Y., Li, Y., Bai, S. and Lin, S. (2010). Tissue deposition and residue depletion in eggs from orally administered animals. Journal of Association of Official Analytical Chemistry International 83(9): 5414-5420.

Bordas, A.C., Brady, M.S., Siewierski, M. and Katz, S.E. (1997). In vitro enhancement of antibiotic resistance development interaction of residue levels of pesticides and antibiotics. Journal of Food Protection 60: 531-536.

Brady, M.S., White, N and Katz S.E. (1993). Resistance development potential of antibiotic/antimicrobial residue levels designated as ‘safe levels’. Journal of Food Protection 56: 229-233.

Cam, Y., Koç A.N., Silici, S., Günes, V., Buldu, H., Onmaz, A.C. and Kasap, F. (2009). Treatment of dermatophytosis in young cattle with propolis and Whitfield's ointment. Veterinary Reclination 11(2): 57-58.

Chukwudebe, A.C., Wislocki, P.G., Sanson, D.R., Halls, T.D. J. and Van den Heuvel, W.J.A. (1994). Metabolism of thiabendazole in laying hen and lactating goats. Journal of Agriculture Food Chemistry 42: 2964-2969.
Mahadeva Naik and Farhath Khanum

Rajashekara, G., Haverly, E., Halvorson, D.A., Ferris, K.E., Lauer, D.C.; and Nagaraja, K.V. (2000). Multidrug-resistant Salmonella Typhimurium DT104 in poultry. *Journal of Food Protection* 63: 155-161.

Rey-Grobillet, X., Eeckhoute, C., Sutra, J.F., Alvinerie, M. and Galtier, P. (1996). Major involvement of rabbit liver cytochrome P4501A in thiabendazole 5-hydroxylation. *Xenobiotica* 26: 765-778.

Reja-Sanchez, A., Ruiz-de-Castaneda, A., Santiago-Laguna, D. and Cano-Marín, M. (1995). Understanding of the health risk associated with presence of antibiotics in cow milk. *Codex Alimentarius* 263: 59-66.

Schaelibaum, M. (1990). Antibiotic therapy and residues in delivered milk. *Swiss Veterinary Journal* 7-9.

Schlegelova, J. (2002). Prevalence of and resistance to antimicrobial drugs in selected microbial species isolated from bulk milk samples. *Journal of Veterinary Medicine* 49: 216-225.

Solomon, E.B., Yaron, S. and Mathews, K.R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied Environment Microbiology* 68: 397-400.

Sci. Technol. Arts Res. J., July-Sep 2014, 3(3): 70-74

Swartz, M.N. (2002). Human diseases caused by food borne pathogens of animal origin. *Clinical Infections Diseases* 34(3): S111-122.

Takahashi, Y., Said, A.A., Hashizume, M. and Kido, Y. (1990). Sulfadimethoxine residue in broiler-chicken skin. *Journal of Veterinary Medical Science* 53: 33-36.

Volkov, I.B. and Kovalev, V.F. (1994). Residual quantity of chemical substances in products of animal origin. *Veterinary Moscow* 4: 42-44.

Wachtel, M.R., Whitehand, L.C. and Mandrel, R.E. (2002). Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *Journal of Food Protection* 65: 18-25.

Willis, C., Booth, H., Westacott, S. and Hawtin, P. (1999). Detection of antibacterial agents in warm water prawns. *Public Health* 2: 210-215.

Wrigley, J., McArthur, M., McKenna, P.B. and Mariadass, B. (2006). Resistance to a triple combination of broad-spectrum anthelmintics in naturally-acquired *Ostertagia circumcincta* infections in sheep. *New Zealand Veterinary Journal* 4(1): 47-49

Yang, T., Huangfu, W.G., and Wu, Y.L. (2011). Melamine residues in eggs of laying hens exposed to melamine-contaminated feed. *Poultry Science* 90(3): 701-704.