DETERMINATION OF TETRACYCLINE ANTIBIOTIC RESIDUE IN DAIRY PRODUCTS SOLD IN OGBOMOSO, SOUTH-WESTERN NIGERIA

*Tona G.O. and Olusola A.D.

Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

*Author for Correspondence

ABSTRACT
This study investigated the levels of the antibiotic tetracycline contained in cow milk, goat milk, butterfat, soft cheese and yoghurt samples. High performance liquid chromatography (HPLC) standard methods were used for the detection and quantification of tetracycline in 40 dairy products samples. All the analysed samples contained residues of tetracycline antibiotics. The mean tetracycline residual levels were as follows: 0.0032 ± 0.0018 ppm (cow milk); 0.0040 ± 0.0011 ppm (goat milk); 0.0020 ± 0.0008 ppm (butterfat); 0.0080 ± 0.0034 ppm (soft cheese) and 0.0019 ± 0.0008 ppm (yoghurt). The level of the tetracycline antibiotic in soft cheese was significantly (P<0.05) higher than those for cow milk, goat milk, butterfat and yoghurt, whose values were similar (P>0.05). The detection of tetracycline residues in all of the tested samples revealed that the processing of cow milk into milk products did not eliminate the tetracycline antibiotic. The observed tetracycline residue levels in the dairy products were below the maximum residue level (MRL) of 0.1 ppm (European Union (EU) standards and were thus safe for human consumption. The government however needs to enforce continuity in the adherence to the tetracycline and other drug maximum residue limits at all times in the dairy industry in Nigeria in order to protect the health of consumers.

Keywords: Tetracycline, Antibiotic Residue, Milk, Milk Products

INTRODUCTION
Milk is the fluid secreted by the mammary gland of mammals for the nourishment of their young ones. Milk and milk products are important sources of animal protein which contribute greatly to human growth and development (Dikko et al., 2010). Milk and milk products are also important products of the Fulani agro-pastoral and other small holder peri-urban systems of Nigeria (Oladunjoye et al., 2001). These authors further stated that in these systems, milk is sold out fresh or processed into other dairy products such as butter, cheese, yoghurt and ice cream in the markets. Tetracycline antibiotic residue was observed to be present in milk products sold out for human consumption in Ibadan, Nigeria (Adetunji, 2008). Antibiotics are widely used in dairy cattle management for the treatment and prevention of diseases and as dietary supplements. They may be administered orally as feed additives or directly by injection. Adetunji (2008) stated that antibiotics find their way into milk through injections or indirectly via the feed. The use of antibiotics may result in drug residues being present in the milk and other dairy products (Phillips et al., 2004). Tetracycline rank among the antimicrobial substances most frequently used in the livestock feed production system (Schmidt and Rodrick, 2003; Al-Mazeedi et al., 2010). In cattle, tetracycline antibiotic can be used when treating general, respiratory, urinary, and local infections (Navratilova et al., 2009). These authors further mentioned that a specific indication for administering tetracycline in cattle is infectious mastitis. A frequent and pervading source of milk contamination with antibiotics such as tetracycline is thus the intra-mammary (intra-cisternal) administration (Schmidt and Rodrick, 2003).

Adetunji (2008) reported tetracycline residue levels range between 0.008 and 0.1µg/ml in milk products in Ibadan, South-western Nigeria, and these values were within maximum residue limits (MRL). Goldfrank et al. (2002) stated that tetracyclines have been reported to cause hypouricemia, hypokalemia, proximal and distal renal tubular acidosis in humans. Also, the presence of antibiotic residues at levels...
higher than the MRL in foods may cause public health hazards including toxicological, microbiological, immunological and pharmacological hazards (Heeschen, 1993). Cerniglia and Kotarski (2005) stated that there could be an emergence of antimicrobial resistance for antimicrobials administered in human therapy, disorders in the intestinal flora and possible occurrence of allergic symptoms. Navratilova et al. (2009) reported that each country within the European Union had established its own surveillance schemes for controlling drug residues through the use of specific rapid testing. However, the high performance liquid chromatography (HPLC) standard methods is widely used for the detection and quantification of tetracycline in milk and milk products (Fritz and Zuo, 2007; Navratilova et al., 2009; Adetunji, 2011).

This study therefore investigated the levels of tetracycline residues contained in raw cow and goat milk and the processed butterfat, soft cheese and yoghurt sold in Ogbomoso, Nigeria. The study also investigated if processing could eliminate or reduce this antibiotic residue in the milk products.

MATERIALS AND METHODS

Sampling Procedures, Processing and Analysis
A total of forty samples (eight samples each) of cow milk, goat milk, butterfat, soft cheese and yoghurt were obtained from a Fulani nomadic farm in Abogunde village, Ogbomoso, Nigeria. The butterfat, soft cheese and yoghurt used were processed from the White Fulani cows’ milk. Twenty mls each of cow milk, goat milk and yoghurt; and 20g each of butterfat and soft cheese samples were put in well labelled, sterile, plastic bottles and kept in a cooler with ice pack and then taken immediately to the laboratory for analysis.

Analysis of Tetracycline Antibiotic Residue
High performance liquid chromatography (HPLC) standard methods were used for the detection and quantification of tetracycline in the milk and milk products samples similar to the methods described by Shaikh and Moats (1993); Clark (1977) and Posyniak et al. (1998) respectively. The HPLC system was equipped with a digital sampler (Model, 616/626 LC) with a fluorescence detector, the column effluent was monitored at a detector wavelength of 280nm.

Preparation of Samples
Five mls of each sample (cow milk, goat milk, butterfat, soft cheese and yoghurt) were put in a 10 ml sterilized pyrex screw cap centrifuge tubes with a mixture of ethanol (5 mls), acetonitril (8 mls), petroleum ether (2 mls) and deionized water (10 mls) solution with pH 3.1. The samples were then centrifuged at 6800 psi and samples were injected into the HPLC columns through the injection valves of size, 2.0 µl. Each column was a stainless packed tube in a stationary phase. The effluent from the columns were then monitored by a fluorescence detector with wavelength of 280 nm. This was a data capture system or personal computer (PC) loaded with software suitable for processing and interpreting of data from the signal of the eluted samples.

Preparation of Working Standard Solutions
Working standard solutions and their readings for the calculation of the standard curves or equations by the software were prepared from tetracycline stock solution (1000 ppm).

Calculation of Results by the Software
A given sample was recorded as positive for tetracycline if its retention time and peak corresponded to that of the working standard. The software generated the standard curves or equations from the signals of the working standard versus the standard concentrations of the unknown analytes. Thus to obtain the concentration of tetracycline in each sample, the reference working standard of known concentration had been injected into the HPLC and the unknown concentration of the sample was extrapolated from the curve peak area. The results were then expressed in ppm.

Statistical Analysis
Samples were analysed in duplicates. The standard deviations of the treatment means were calculated. All statements of differences were based on significance at P<0.05.
RESULTS AND DISCUSSION

Levels of Tetracycline Antibiotic Residue in Dairy Products Samples

All the 40 dairy products samples analysed had detectable levels of tetracycline antibiotic residue and the range was 0.0019 to 0.0080 ppm as presented in Table 1 and Figure 1. The range of values of 0.0019 to 0.0080 ppm obtained in this study was lower than 0.01 to 0.10 ppm tetracycline residue levels reported by Adetunji (2008) for milk and milk products. The occurrence of tetracycline antibiotic residue in all the samples might be attributed to factors such as milking of animals at the time of tetracycline antibiotic treatment and probably the over dose of the antibiotic used.

Table 1: Levels of tetracycline antibiotic residue in dairy products samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean concentration of tetracycline (ppm)</th>
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<tbody>
<tr>
<td>Cow milk</td>
<td>0.0032 ± 0.0018&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Goat milk</td>
<td>0.0040 ± 0.0011&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butterfat</td>
<td>0.0020 ± 0.0008&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soft cheese</td>
<td>0.0080 ± 0.0034&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>0.0019 ± 0.0008&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation;<sup>a,b</sup> values with different superscripts along the column are significantly (P<0.05) different.

The mean concentration of tetracycline antibiotic residue in dairy products samples was significantly (P<0.05) higher in the soft cheese samples with the value of 0.0080 ± 0.0034 ppm than in other samples. Mean levels in other samples were as follows: 0.0032 ± 0.0018 ppm (cow milk), 0.0040 ± 0.0011 ppm (goat milk), 0.0020 ± 0.0008 ppm (butterfat) and 0.0019 ± 0.0008 ppm for yoghurt. These values were not significantly (P>0.05) different one from another as presented in Table 1. The butterfat, soft cheese and yoghurt samples were processed from the cow milk of White Fulani cows and the heat treatment during processing did not eliminate the tetracycline antibiotic residue. Tetracycline antibiotic residue level in cow milk (0.0032 ± 0.0018 ppm) was not significantly (P>0.05) different from that of goat milk (0.0040 ± 0.0011 ppm). The significantly (P<0.05) higher level of tetracycline antibiotic residue in cheese samples (0.0080 ± 0.0034 ppm) in the current study was contrary to the report of Adetunji (2011) who observed a lower range of 0.0011 to 0.0042 ppm of tetracycline residue levels along cheese processing lines from the time of addition of coagulant to curdling point and then to cheese mould stages. This higher level of the antibiotic in cheese samples in this study could be due to improper pasteurization before the addition of coagulant during processing.
Adetunji (2008) mentioned the observation that proper pasteurization could be of help in reducing antibiotic residues to the barest minimum levels during cheese processing. The finding that the heat treatment or processing of cow milk into other milk products (butterfat, soft cheese and yoghurt) neither eliminated nor reduced the antibiotic residue, was in agreement with the reports of previous researchers (Adetunji, 2008; O’Keefe and Kennedy, 2008), who also recorded very little or no effect on tetracycline antibiotic residue after processing milk into other milk products. Furthermore, the presence of tetracycline antibiotic residue in the dairy products analysed in this study could also be attributed to the thermo-resistant nature of tetracycline antibiotic as stated by Robinson (1987) and Anon (1998). Similarly, Navratilova et al. (2009) confirmed that heat treatment of milk only brought about a partial reduction of tetracycline antibiotic residue concentration and that total elimination did not take place.

There is paucity of information on compared tetracycline residue concentration values in the milk of different species of ruminants such as between cow milk and goat milk. In the present study there were non significant (P>0.05) difference between the tetracycline antibiotic residue levels in cow milk (0.0032 ± 0.0018 ppm) and goat milk (0.0040 ± 0.0011 ppm). These probably showed that the residual effect of the tetracycline antibiotic was similar in raw milk, irrespective of the species of lactating ruminant livestock. Zhao et al. (2004) found higher tetracycline concentration range of 0.013 – 0.106 ppm in cow milk in the USA, which was just within the recommended 0.1 ppm MRL (FDA, 1996; Folly and Machado, 2001).

Maximum Residue Level for Tetracycline in Cattle Milk

Table 2 shows the maximum residue level for tetracycline in cattle milk. Thus the concentrations of tetracycline residue in dairy products that exceed the 100 ppb/0.1 ppm MRL is prohibited by the European Union (EU) and the Code of Federation Regulations (CFR) of Kuwait. In the current study, the tetracycline antibiotic residue levels were all below the MRL of 0.1 ppm stated in Table 2.

<table>
<thead>
<tr>
<th>EU MRL</th>
<th>CFR MRL (Kuwait)</th>
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<tr>
<td>100 ppb/0.1 ppm</td>
<td>100 ppb/0.1 ppm</td>
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(CFR = Code of Federation Regulations; MRL= maximum residue limit)

Conclusion

This study showed that tetracycline antibiotic residue was detected in all of the dairy products samples analysed. Also the processing of cow milk into milk products did not eliminate the tetracycline antibiotic. This residue was however present at levels below the maximum residue limit (MRL) of 0.1 ppm. The government however needs to enforce continuity in the adherence to the tetracycline and other drug maximum residue limits at all times in the dairy industry in Nigeria in order to protect the health of consumers.

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