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DETERMINATION OF AFLATOXIN LEVELS MIXED CATTLE FEEDS FROM DIFFERENT FARMS IN DIYARBAKIR REGION

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Abstract: This study aimed to determine the levels of aflatoxins in beef and dairy cow supplied by feed manufacturing plants in the Diyarbakır region, accounting for a significant proportion of cattle production in Southeast Anatolia. In the study, Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) levels were determined in dairy cattle feed and fattening cattle feed. The toxic effects of aflatoxins on animal organisms are listed as AFB1, AFG1, AFB2, and AFG2 from largest to smallest. Regional feeds were evaluated based on the limits of AFB1 (≥0.005 ppm) in dairy cow feed and AFB1 (≥0.02 ppm) in cattle feed. By the Regulation on Unwanted Substances in Feeds (Regulation No: 2014/11), contamination values were considered in terms of total aflatoxin levels in feeds. According to the determined mycotoxin contamination levels, the average aflatoxin level in cattle milk feed was 0.0036 ppm and in cattle feed was 0.0034 ppm. This study emphasizes the importance of storage conditions and preservation methods of feeds and raw materials.

Keywords: Aflatoxin, Cattle feeds, Contamination

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1. Introduction

Mycotoxins are secondary metabolites produced mainly by several fungal species, including *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium*. The Food and Agriculture Organization (FAO) has reported that 25% of the world's food is contaminated with mycotoxins (Nazhand et al., 2020). However, a recent study has shown that 60-80% of crops worldwide are contaminated with mycotoxins, exceeding the figure reported by the FAO (Escola et al., 2020). Furthermore, in 2016, the World Health Organization reported that more than 20% of the global burden of disease and death is preventable and attributable to environmental factors, including both environmental and genomic risk factors, as well as exposure to AFB1 and AFM1 (Joubert et al., 2020).

The mycotoxins produced by *Aspergillus* spp. are known as aflatoxins. The genus *Aspergillus* comprises four subgenera and 339 species (Campione et al., 2020). Aflatoxins are mainly produced by *Aspergillus flavus* and *A. parasiticus*, but some other species from the Flavi section such as *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, and *A. bombycis* have also been reported as aflatoxin producers. In addition, species from the Ochraceorosei section such as *A. ochraceoroseus* and *A. rambellii*, and the Nidulatans section such as *Emericella astellata* and *E. venezuelensis*, are known to produce aflatoxins (Ahmad et al., 2014). Several types of aflatoxins have been identified, and their contamination of economically important crops and foods is a major global concern (Luo et al., 2021). They are both carcinogenic and mutagenic. These substances are extremely harmful and carcinogenic, inflicting illness on both people and livestock. The most common toxin, aflatoxin B1, is present in plant substrates and has the highest potential for toxicity.

According to Chen et al. (2013), aflatoxins are stable tiny molecules that cannot be eliminated by heat treatment or during processing.

They cause aflatoxicosis in both humans and animals (Kumar et al., 2017) and more than 20 metabolites of the aflatoxin group have been identified in feed and food (Mahmood Fashandi et al., 2018). Given the detrimental impact of aflatoxins on biological systems, the European Commission and the US Food and Drug Administration (FDA) have established maximum permissible levels for these toxins in food and feed products at 20 parts per billion (ppb). The European Union has set a more stringent limit of 4 ppb (Cheli, F et al. 2014; FDA, 2019; Kaale, 2021).The contamination of human foods and animal feeds by toxic substances, known as mycotoxins, represents a significant risk. Mycotoxins are harmful chemicals produced by mold fungi. The most well-known and researched mycotoxins are aflatoxins. As toxic

secondary metabolites, mycotoxins are produced by specific fungi belonging to *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* species. Agricultural products could become contaminated by these mycotoxins at several points in the food supply chain. (Uçkun and Var 2014; Gbashi et al., 2020; Areo et al., 2023)

Crops and their byproducts frequently contain aflatoxins (B1, B2, G1, and G2). Furthermore, animal byproducts, especially milk and other dairy products, are the main source of AFM1 and AFM2, which are metabolites of AFB1 and AFB2 (Khan et al., 2021; Ye et al., 2023). Aflatoxins are toxic substances that are resistant to the temperatures typically employed in milk processing techniques. Complete breakdown of the aflatoxins occurs at 300°C; however, processes such as sterilisation, UHT (ultra-high temperature) and pasteurisation are insufficient for the elimination of aflatoxins (Alçiçek, 2012). Aflatoxins can be eliminated through the use of sunlight, ultraviolet (UV) rays, and gamma rays. Of these methods, gamma rays have been identified as the most effective (Yaroğlu, 2007).

Aflatoxins are classified into six distinct types: B1, B2, G1, G2, M1, and M2. They are a significant threat to human and animal health. These toxins are heat-resistant and have been linked to an increased risk of cancer, genetic mutations, and birth defects. Aflatoxin poisoning (aflatoxicosis) can occur in lactating animals when they are fed moldy feeds, including mixed feed, roughage, pulp, and silage (Okechukwu et. al., 2024). According to Ye et al. (2023), *A. flavus* primarily produces AFB1 and AFB2, while *A. parasiticus* synthesizes AFB1, AFB2, AFG1, and AFG2. Additionally, a wide range of foods contain AFB1 and AFB2, including spices oilseeds, cereals (including maize, sorghum, pearl millet, rice, and wheat), fresh and dried fruits , vegetables, juices, and dairy products, as noted by Massomo (2020). It is therefore of great importance to control the presence of aflatoxins in milk and dairy products in order to safeguard human and public health. They have a detrimental impact on the quality of feed and food safety, and are a significant hazard to human and animal health (İpçak and Alçiçek, 2013). In 2016, the World Health Organization reported that more than 20% of the global disease burden and deaths are attributable to modifiable environmental factors. Furthermore, exposure to AFB1 and AFM1, which are associated with both environmental and genomic risk factors, is also evaluated within these groups (Joubert et al., 2020). As a consequence global climate change, aflatoxin has emerged as a threat in regions that were previously free from this hazard (Jallow et al., 2021).

In recent years, there has been a growing interest in the nutritional value and mycotoxin levels of feeds used in ruminant nutrition in our country. Our farmers are seeking further information on this matter and are aiming to improve the suitability of these feeds for ruminants. The appropriate techniques must be followed at each stage of the process, from preparation and transportation to storage and delivery, to ensure the quality and safety of the feed for the animals. The results of this study have demonstrated the necessity for measures to be taken to safeguard the health of animals and humans.

2. Materials and Methods

2.1. Materials

The study consists of 20 samples of cattle fattening feed and 20 samples of cattle dairy feed. The samples were collected in sealed and sealed containers for inspection from feed factories and smaller-scale feed production enterprises and farms located in the provinces of Diyarbakır, Mardin, Siirt, Batman, Şırnak, and Bingöl provinces, as well as the districts connected to these provinces, and were sent to the Diyarbakır Food Control Laboratory Directorate in the region. The objective of the study was to ascertain the levels of aflatoxins present in the feeds consumed in the region. Prior to commencing the analytical procedures, the samples were subjected to grinding and subsequent homogenisation through a 1 mm sieve, in order to ensure uniformity.

2.2. Method

In this study, feed samples obtained from the designated enterprises were analyzed for aflatoxins according to the A.O.A.C. Official Method (2003). Aflatoxin B1 in Cattle feed this study employed the immunoaffinity column and column derivatisation feature of reverse-phase, high-performance liquid chromatography (HPLC) for the determination of Aflatoxin B1 in feed, feed raw materials, and feed additives where its presence is undesirable.

2.2.1. Preparation of HPLC mobile phase

An Ultra-Pure Water: Acetonitrile: MeOH (6:2:3/v:v) mixture was prepared. To this solution, 119 milligrams of Potassium Bromide and 350 microliters of 4M HNO_3 were added per liter of the solution. Prior to utilisation, the solution was filtered through a 0.45 µm glass microfiber filter paper and stored in the dark at 2 to 8 °C. Calibration standards were prepared based on the certified values of the standards to be used in the study.

2.2.2. Extraction

Twenty-five grams of the homogenized sample were weighed and placed in a container. Subsequently, 125 mL of the solvent (85% acetone + 15% distilled water, v/v) was added to the sample, which was shaken for 60 minutes. Afterward, the mixture was filtered through Whatman filter paper, and 5 mL of the filtrate was pipetted out. To this collected filtrate, 95 mL of distilled water (dH_2O) was added and thoroughly mixed to dilute the extract. Finally, the diluted extract was filtered through a glass microfiber filter.

Immunoaffinity column (IAC)

An immunoaffinity column (IAC) was connected to a syringe with a reservoir via a Luer-lock syringe tip, which was in turn attached directly directly attached to a vacuum manifold. In order to condition the column, 20 ml of PBS was passed through the immunoaffinity column. Subsequently, 50 ml of the diluted and filtered extract was passed through the immunoaffinity column

(IAC), and adjusted to flow at a rate of one drop per second. After all the extract had passed through the IAC, a wash step with 20 ml of distilled water (dH_2O) or PBS was performed to wash the column. After completing these steps, the air was passed through the column using a piston to ensure the complete removal of liquid from the column.

The eluate was collected into a 6 ml vial from the immunoaffinity column (IAC). Subsequently, 1.75 mL of HPLC-grade MeOH was added into the IAC to facilitate elution from the column. After the MeOH completely passed through the IAC, some air was passed through to help transfer all the MeOH into the vial. To the eluate collected in the vial, 3.25 mL of distilled water (dH_2O) was added to facilitate mixing, and it was filtered through a 0.2 µm filter. Following these procedures, the liquid collected in the vial, prepared according to HPLC chromatographic conditions, was thoroughly mixed in a vortex mixer. Subsequently, 100 µl of the sample was taken and injected into the instrument (HPLC) for analysis.

2.2.3. HPLC injection

The high-performance liquid chromatography (HPLC) instrument used has an excitation wavelength (λ Ex) of 360 nm and an emission wavelength (λ Em) of 440 nm, operating at a temperature of 25 °C. The pump flow rate is set at 1 ml per minute with a pressure of less than 300 bar. The injection volume is 100 microliters.

Following the injection of the sample into the HPLC instrument, the obtained results are evaluated by calculating the dilution factor. The analytical results are automatically obtained based on a prepared calibration curve. The reported result takes the form of a ± Ux after correction for dry matter and recovery rate from the raw data.

In these analytical studies, an HPLC instrument was utilized, and statistical calculations were performed using the SPSS 9.0 (1999) software.

3. Results

In the study, analyses of cattle dairy feeds revealed that out of 20 samples, 16 samples were found to have aflatoxin levels below 0.005 ppm, while 4 samples exceeded 0.005 ppm. The highest concentration of aflatoxin detected in the dairy feed samples was 0.02 ppm, while the lowest was below the instrument's limit of quantification (LOQ), measured at 0.00022 ppm.

All 20 samples of cattle fattening feedwere found to be below the legal limit of 0.02 ppm. The highest concentration of aflatoxin was observed in cattle fattening feed at 0.01478 ppm, while the lowest concentration was 0.00022 ppm.

Based on the aflatoxin (AF) results obtained from the analysis of dairy cattle feed (Table 1), the significance of AF levels can be summarized as follows: the highest AF level measured was 0.018 ppm. The results of this analysis indicatethat the AFB1 content in the dairy feeds may potentially be high. Aflatoxin B1 can have serious effects on animal health, and such high levels can lead to toxic effects in animals. In the study, AFB2, AFG1, and AFG2 levels were also measured, and the results generally showed low levels or were not detected at all. These can also have adverse effects on animal health, although not as pronounced as AFB1. High levels of aflatoxins found in feeds (values below 200 ppb) have been reported to potentially lead to chronic poisoning, even though clinical acute and subacute symptoms are rare at lower levels. The establishment of limits is due to the suppression of the immune system in animals, changes in the biochemical structure of blood (disturbances in protein synthesis involved in clotting), and damage to cells in the liver and other tissues, especially the bile ducts (Kaya, 2002).

Table 1. Aflatoxin B1 (AFB1) and Aflatoxin B2 (AFB2) levels in cattle dairy feed (ppm)

Cattle dairy feed	AFB1 (ppm)	AFB2 (ppm)
N	20	20
Mean	0.00323540	0.00039540
Median	0.00196400	0.00012700
Std. Deviation	0.004082365	0.000594435
Range	0.017879	0.002155
Minimum	0.000222	0.000000
Maximum	0.018101	0.002155

Table 2. Levels of Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) in cattle dairy feed (ppm)

Cattle dairy feed	AFB1 (ppm)	AFB2 (ppm)
N	0.00000925	20
Mean	0.00000000	0.00000000
Median	0.000041367	0.00000000
Std. Deviation	0.000185	0.000000000
Range	0.000000	0.000000
Minimum	0.000185	0.000000
Maximum	0.00000925	0.000000

Table 3. Total aflatoxin levels in cattle dairy and fattening feed (ppm)

Cattle dairy feed	Cattle Dairy Feed Total Aflatoxins (ppm)	Cattle Fattening Feed Total Aflatoxins (ppm)
N	20	20
Mean	0.00364000	0.00347600
Median	0.00211250	0.00225500
Std. Deviation	0.004644885	0.003682625
Range	0.020035	0.014560
Minimum	0.000222	0.000220
Maximum	0.020257	0.014780

Table 4. Cattle fattening feed AFB1 and AFB2 levels (ppm)

Cattle fattening feed	AFB1 (ppm)	AFB2 (ppm)
N	20	20
Mean	0.00475700	0.00032900
Median	0.00205000	0.00019500
Std. Deviation	0.008475318	0.000356207
Range	0.037780	0.001170
Minimum	0.000220	0.000000
Maximum	0.038000	0.001170

Table 5. Cattle fattening feed AFG1 and AFG2 levels (ppm)

Cattle fattening feed	AFB1 (ppm)	AFB2 (ppm)
N	20	20
Mean	0.00002800	0.00021300
Median	0.00000000	0.00000000
Std. Deviation	0.000125220	0.000952565
Range	0.000560	0.004260
Minimum	0.000000	0.000000
Maximum	0.000560	0.004260

In our country, maximum acceptable levels for aflatoxins (B1, B1+B2+G1+G2) have been established for animal feed products considered undesirable substances in feeds. Accordingly, the highest amount detected in feed ingredients is 0.02 mg/kg (ppm), based on a feed containing 12% moisture. In accordance with the Regulation on Feedstuffs (TGK, 2011), the maximum permitted level for mixed feeds for dairy cows and calves, dairy sheep and lambs, and dairy goats and kids is 0.005 mg/kg.

AFB2, AFG1, and AFG2 levels were also measured (Table 2), but they are generally found at low levels or were not detected. These can also have adverse effects on animal health, although not as pronounced as AFB1. AFB2, AFG1, and AFG2 levels were also measured, but they are generally found at low levels or were not detected.

The total aflatoxin level (Table 3) in the highest sample is 0.02 ppm. This indicates a generally high aflatoxin content in the dairy feeds. Total aflatoxin levels refers to the sum of various aflatoxin types, and high levels can cause serious harm to animals. The amount of AF contamination not only reduces the value of cereals used as animal feed, but has also been associated with increased mortality rates in livestock (Massomo, 2020; Khan et al., 2021). In a study conducted by Yıldırım et al. (2018), research on feed and milk samples taken from dairy farms in Kırıkkale between 2012 and 2013 revealed that aflatoxin (AF) and aflatoxin M1 (AFM1) were detected in all 154 samples.

In their study, Yıldırım et al. (2018) observed a range of aflatoxin (AF) levels in dairy feed ranging from a minimum of 0.20 ppb to a maximum of 28.80 ppb, with an average of 6.43±7.01 ppb. In a study conducted in Erzurum (Polat, 2012), the presence of AFB1 in feed ingredients used in dairy cattle operations and AFM1 in milk was investigated.

In this study, the lowest level of AFB1 in feed sources was found to be 1.89±0.34 ppb, while the highest AFB1 level was 3.29±0.59 ppb (Polat, 2012). The levels of AFB1 and AFB2 (Table 4) are generally fall within the acceptable limits. Both mycotoxins did not show any adverse effects.

The levels of AFG1 and AFG2 were either not detected or found at very low levels (Table 5). This indicates that these mycotoxins pose no potential risk to cattle.

In the analysis of 20 samples of cattle fattening feed, total aflatoxin levels ranged from a minimum of 0.00022 ppm to a maximum of 0.014 ppm. In accordance with Regulation on Undesirable Substances in Feeds (Regulation No: 2014/11), no results above ≥0.02 ppm were identified.

The total aflatoxin levels are generally found to be low, indicating overall good feed quality. Based on the aflatoxin results obtained from the analysis of cattle feeds, the importance of aflatoxin levels can be summarized as follows. High levels of aflatoxins can adversely affect the health, productivity, and milk quality of cattle. Animals consuming feed contaminated with AFs may exhibit a variety of symptoms such as weakened immune function, developmental delays and complications associated with malnutrition, but the risk varies depending on factors such as age, species and individual susceptibility. Acute aflatoxicosis in animals can manifest as depression, weight loss, liver damage and gastrointestinal haemorrhage, and in severe cases can lead to death (Navale et al., 2021) Prolonged exposure to AFs can inhibit the growth rate of young animals and reduce the quality of milk and egg production (Ingle et al., 2020).

Translated with www.DeepL.com/Translator (free version) regular monitoring of aflatoxin levels in dairy feed is critical to prevent excessive exposure of animals to these toxins. If the levels of aflatoxins exceed a certain threshold, it's important to reduce the use of such feeds or switch to alternative feed sources. Storage conditions and hygiene measures for feeds should also be reviewed.

4. Discussion

These findings emphasise the necessity for measures to be taken in order to protect the health of cattle and to guarantee the quality of milk. In light of the findings of this study, it is recommended that feed samples be subjected to regular analysis and that feed sources be diversified. It is possible to improve the nutritional balance of cattle and reduce the risk of mycotoxin contamination by providing them with diverse nutrients. Information related to mycotoxin contamination, particularly focusing on aflatoxins and their impact on animal and human health, is of interest. According to data in the RASFF (Rapid Alert System for Food and Feed) database in (2020), aflatoxin contamination was reported in various food ingredients such as pillows, rice, nuts (pistachios, hazelnuts, and almonds), spices, and dried figs. These contaminations occurred up to 1000 µg/kg in some samples.

The high concentration levels are claimed to have arisen from inadequate food management practices during the COVID-19 pandemic worldwide. As a result, there is an expectation of increased consumption of aflatoxin-contaminated food by both animals and humans, potentially leading to an increase in associated health problems (Pickova et al., 2021)

Feed storage conditions should be monitored regularly, and an appropriate storage environment should be maintained. The results of this study are critical in evaluating feeding practices in the broader context of overall cattle health and productivity. By consulting local veterinarians or livestock experts, the cattle feeding program can be optimized. Aflatoxin levels in cattle feed can significantly impact the health, performance, and quality of cattle products. Aflatoxins are natural toxins produced by mold fungi and can occur particularly in corn, peanuts, cottonseed, and other agricultural products. If these feeds are given to animals, aflatoxin exposure can lead to various health problems. High levels of aflatoxins can cause liver damage, digestive issues, weakened immune systems, and reproductive problems in cattle. The accumulation of aflatoxins in animals can lead to long-term health issues (Aydın, 2007).

Aflatoxin levels can negatively affect the productivity and performance of cattle. This can result in reduced growth rates, reduced milk yield, and deterioration in meat quality. In conclusion, aflatoxins pose a threat to both human and animal health. Various measures can be taken to reduce the toxicity of aflatoxins, but the most effective method is the complete removal of aflatoxins from food (Özkaya et al., 2003).

Cattle cannot develop resistance to high levels of aflatoxins. Continued feeding of aflatoxin-contaminated feed can lead to long-term health problems. Specifically, the accumulation of aflatoxins in milk and meat from dairy cattle can adversely affect consumer health and limit the trade of these products. For these reasons, it is important to regularly monitor and control aflatoxin levels in cattle feed and to take appropriate action. Feed suppliers, farm owners, and veterinarians must be vigilant to protect the health and productivity of the animals.

According to meteorological data from 1929 to 2022, the temperature in the region ranged from 1.8 to 31 °C, and the relative humidity ranged from 47.1% to 62.7%. This indicates that the conditions for aflatoxin production in cattle feed were not suitable, which is reflected in the results. Although the aflatoxin limits for dairy cattle feed are four times lower than for fattening feed, only 20% of the tested samples exceeded the legal limits. Mycotoxins are a threat in our country, especially in tropical, subtropical and temperate regions. High humidity in coastal areas is thought to be more conducive to the growth of mycotoxins. Delayed harvesting increases the likelihood of AF production and is also conducive to fumonisin production (Mansfield et al., 2007; Atukwase et al., 2009; Da Costa et al., 2018).

Abiotic factors such as temperature, water activity, pH, carbon, and nitrogen significantly affect the biosynthetic pathway of aflatoxins (Abdel-Hadi et al., 2012; Liu et al., 2017; Tejero et al., 2021). Many analytical methods have been developed for the detection of aflatoxins. Instrumental methods such as enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography with fluorescence detection (HPLC-FLD) (Sheijooni-Fumani et al., 2011; Kong et al., 2013), and ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Scholl and Groopman, 2008; Deng et al., 2018; Tonbak and Demir, 2021) are commonly used for aflatoxin detection.

Most countries and organizations have established legal

regulations to limit the levels of mycotoxins, mainly aflatoxins, in order to minimize problems that may arise from mold contamination (Li et al., 2020). Also an another study conducted that, water activity and temperatures have a complex influence on the regulation of genes involved in A. flavus growth and aflatoxin biosynthesis (Liu et al., 2017)

Control of mold growth in feed is essential to prevent contamination with all mycotoxins, including aflatoxins. This includes preventing contamination during the cultivation, harvesting, storage, transport of raw materials, and processing of products. In feed ingredients, various physical, chemical, and biological methods are being tested to prevent and remove contamination. Additionally, the use of different biotransformers such as microorganisms and their purified enzymatic products in feeds can lead to the catabolism, breakdown, or conversion of aflatoxin molecules into non-toxic metabolites.

Similarly, various clay materials such as bentonite, hydrated calcium aluminosilicate (HSCAS), zeolite, and activated charcoal have been shown to reduce AF levels in contaminated feeds. However, to date, different techniques are being applied to prevent or reduce AF formation. These techniques encompass physical, chemical, biological, enzyme, amino acid, and vitamin-based methods. Treatment with alkaline compounds, in addition to certain other salts and acids such as hydrochloric acid, phosphoric acid, sodium, potassium, calcium hydroxide, sodium bicarbonate, sodium chloride, and sodium sulfate, has been shown to reduce aflatoxin contamination by 18-51%.

The successful use of ozone and chitosan nanoparticles has been demonstrated in reduce aflatoxin levels. Sodium hydrosulphide, when applied in the range of 0.25-2%, provides a reduction of 96-100% (Sipos et al., 2021). Among the most studied methods are those based on the use of biological adsorbents, mainly bacteria and yeast (Atasayar et al., 2008; Giovati et al., 2015). It is evident that aflatoxins pose a threat to both human and animal health. Various measures can be taken to reduce the toxicity of aflatoxins, but the most effective method remains the complete removal of aflatoxins from foods (Özkaya et al., 2003).

5. Conclusion

Studies have demonstrated that contamination of milk and dairy products with AFM1 can be considerable, with levels that could potentially pose a threat to public health being reached. The importance of AFB1 and AFM1, which are associated with various viruses and cancers in humans and animals, is increasingly recognized due to productivity losses. Progress is being made in methods for their elimination. With advancements in analysis and elimination techniques, strict adherence to protocols for the prevention and control of AFB1 in feed and raw materials, as well as AFM1 in milk and dairy products should continue. Therefore, it is beneficial to educate and raise awareness among individuals and organizations involved in the production, storage, and distribution of feeds, various foods, as well as milk and dairy products, on both national and international platforms. This can be achieved through a variety of activities aimed at informing and raising awareness among producers and consumers alike.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.E.	A.B.K.
C	50	50
D		100
S		100
DCP	100	
DAI		100
L	50	50
W	50	50
CR	50	50
SR		100
PM	20	80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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