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Author's Affiliation:
1. Department of Veterinary Public Health, College of Veterinary Medicine, Baghdad University – Iraq
2. Avi-Cenna Elearning Center, Baghdad University – Iraq

***Corresponding Author:**
Huda Nsaif Jasim
Email: hoda.nj@covm.uobaghdad.edu.iq

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Detection of Glyphosate in Sheep Feed and Drinking Water Collected Randomly from Various Agriculture Areas Located in Different Territories of Baghdad Province

Raad Jabbar Hammadi[1], Huda Nsaif Jasim[1]*, Mohammed Sadeq Salman[2]

Abstract

Background: The primary objectives of this study were to employ a quantitative method, specifically High-Performance Liquid Chromatography (HPLC), for the purpose of detecting glyphosate residues (measured in parts per million) in the feed and drinking water consumed by sheep.

Methods: During the period from February 2021 to May 2021, a sample of 30 feed and drinking water samples from sheep was collected in a random manner. These samples consisted of 15 feed samples and 15 drinking water samples. The collection was conducted in various agricultural areas situated in different territories within the province of Baghdad.

Results: The HPLC residues study showed that 12 and 7 samples, respectively, had glyphosate residues in sheep feed and drinking water. Of the 15 feed samples analyzed, 12 were positive for glyphosate residues: 5 alfalfa, 4 barley, and 3 clover samples. Alfalfa, barley, and clover had the greatest glyphosate residues (140.5–145.5, 120.7–139.5, and 119.5–128.2 ppm), respectively. HPLC analysis showed that all forage samples—alfalfa, barley, and clover—exceeded the Maximum Residue Levels (MRLs) (0.05, 20, and 0.05), violating the EFSA's 2019 recommendation for glyphosate (ppm) in sheep feed. Alfalfa showed the most significant violation, with residue levels over 2,800-fold the Maximum Residue Level (MRL), followed by clover (over 2,400-fold) and barley (6.5-fold). Sheep's Water (Barn, Field, and Drained) samples (5 of each) were positive for glyphosate residues (2, 3, and 2), with the highest ranges in Field (104.1–106.3), Barn (83.7–85.5), and Drained (0.3–0.5). HPLC analysis showed that all water samples except Drained water (Barn and Field) above the Maximum Contamination Level (MCL) (0.7), violated the EPA's glyphosate (ppm) recommendation. HPLC analysis showed that glyphosate levels in Barn and Field water were dramatically above the MCL, exceeding the EPA's recommendation by over 120-fold and 150-fold, respectively.

Conclusion: The utilization of glyphosate has exhibited a progressive rise over the course of time, thereby eliciting apprehensions regarding the potential toxicity of this herbicide as well as its plausible impact on human health. Glyphosate residues have the potential to be transmitted to humans or animals via water, feed, and food that has been contaminated.

Introduction

Over the past 25 years, glyphosate use has multiplied more than ten times throughout the world [1]. Roundup Ultra is a general non-selective glyphosate herbicide that was used widely by Iraqi farmers [2]. Residues of glyphosate have persisted in water and soil bodies due to considerably slower breakdown than initially predicted. As a result, there are now more of these residues in animal and plant products [3,4].

Different mechanisms exist through which glyphosate can penetrate nontarget plants, such as spraying, released via the tissue of plants that have been treated, and weeds' dead tissue. Residues of glyphosate are being found in the food and feed of many different items as a result of this non-target exposure [5]. In order to ensure the health of consumers and facilitate international commerce, regulatory measures have been established to establish maximum residue limits (MRLs) for pesticide residues in various food and feed products derived from plants and animals. These MRLs are outlined in the (EC) Regulation 396/2005 and are endorsed by relevant authorities [6,7].

Alfalfa, clover and barley are important forage crops behind corn, soybeans, and wheat used around the world and Weeds significantly affect the protein content and general quality of feed by changing the composition of these crops. To guarantee a successful plant population during stand establishment, weed control is essential. Up to five days before harvest, glyphosate can be applied once more if weed issues continue. To get rid of the small proportion of glyphosate-vulnerable forage plants that exist in fresh seeding, glyphosate must first be applied [8,9,10].

Following application, glyphosate is taken up by the foliage and distributed throughout the roots leaves and stems of the plant. Pesticides are not significantly broken down or metabolized in sensitive plants. The 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), An enzyme necessary for the process of aromatic amino acid metabolism, is what gives herbicides their herbicidal effects. A reduction in the synthesis of protein caused by the inhibition of amino acid metabolism stops growth, disrupts cells, and kills plants.

However because the herbicide does not inhibit the enzyme EPSPS in crops of Roundup Ready (RR), the crops are not harmed there to maximize the benefits farmers used RR extensively. It's been established that, following the consumption of herbicides, in all areas of soybean plants, translocation takes place and the primary byproduct of the metabolism of glyphosate in leaves is aminomethyl phosphonic acid (AMPA) [11].

A broad-spectrum pesticide with widespread application is glyphosate. In fact, it's a herbicide. that is used the most on all feed crops [12, 13].

There are several ways that glyphosate can contaminate surface water, mainly by drifting during spraying or as runoff from the surface after application. Other sources of glyphosate in waters open include wastewater treatment facility outputs, Spray from the wind comes from nearby treated regions, and direct weed-control spraying on open water. According to reports, metabolite aminomethylphosphonic acid AMPA and glyphosate levels on water's surface contaminated by glyphosate may vary starting from (sub-μg/L) to (mg/L) values [14]. International standards stipulate that permitted amounts of glyphosate in water vary considerably by nation, with the (EU) permitting a level maximum of (0.1 mg/L) for pesticides on water of drinking [15].

Fortunately, glyphosate content within the water of the surface is influenced by a number of environmental variables, including the subsequent rain events and the composition of the soil. These elements may result in more glyphosate reaching surface waterways [16].

The most widely used herbicides contain glyphosate, with Roundup (RUP) and related residuals are frequently contaminating water on the surface, groundwater, and food [17]. The primary objectives of this research were to utilize a quantitative approach, namely High-Performance Liquid Chromatography (HPLC), to detect glyphosate residues (measured in parts per million) in the feed and drinking water ingested by sheep.

Methods

Samples collection

A total of 30 sheep feed and drinking water samples (15 samples of each) were collected randomly from various marketplaces and agriculture areas located in different territories of Baghdad province from February 2021 to May 2021. These samples were analyzed by using High-Performance Liquid Chromatography Techniques (HPLC).

Preparing samples for the detection of glyphosate

Each sample was contained individually in a sterile glass container also wrapped in aluminum foil to protect it from degradation by light and identified with a label then transported to the laboratory.

In a 100 mL centrifuge tube made of polypropylene, 20 g of feed (alfalfa, barley, and clover) samples were placed, along with 10 mL of water that had been distilled. The samples were extracted with the addition of (10 ml) of acetonitrile then vigorously shaking for (1 min.). The combination was vigorously agitated and centrifuged for phase separation after the addition of salts (0.5 ± 0.03 g) disodium hydrogen citrate sesquihydrate, (1 ± 0.05 g) trisodium citrate dehydrate, (1 ± 0.05 g) sodium chloride and (4 ± 0.2 g) magnesium sulfate anhydrous).

A minimum of (8 ml) from the extraction was put into a (15 ml centrifuge disposable tube) and put in the freezer for (1 hr). After the extraction had almost completely thawed, (6 ml) of it was moved to a 15 ml (15 ml centrifuge disposable tube) that also contained 900 mg of MgSO4 and 150 mg of primary secondary amine. This tube was used for clean-up to get rid of interference and lessen instrument contamination. The finished extract was centrifuged once more after which it was put into a storage vial with a screw-capped and then kept in a freezer until examination.

Statistical analysis

With the help of SAS (Statistical Analysis System - version 9.1), data were statistically analyzed. To determine whether there were significant differences between the means, one-way, two-way (ANOVA), or analysis of variance as well as the Least Significant Differences (LSD) post hoc test were used.

To evaluate the percentage of differences that are significant, a chi-square analysis was also performed. Statistics are considered significant if ($P<0.05$)[18].

Results

Glyphosate residues in Feed samples

The HPLC residues analysis revealed positive glyphosate residues in 12 feed samples: 5 alfalfa samples, 4 barley samples, and 3 clover samples. The highest ranges of glyphosate residues were recorded in alfalfa followed by barley then clover, (140.5-145.5), (120.7-139.5) and (119.5-128.2) respectively with the highest mean±SE of glyphosate residues were recorded in alfalfa followed by barley then clover, (143.26±1.01), (130.10±5.42) and (124.46±2.58) respectively.

Forage samples	No. of total samples	NO. of positive samples	Range	MRLs (ppm)	Means±SE	Violation (Fold increase over MRL)
Alfalfa	5	5	140.5-145.5	0.05	143.26±1.0	287
barley	5	4	120.7-139.5	20	130.10±5.42	6.5
clover	5	3	119.5-128.2	0.05	124.46±2.58	248

Table 1: Level of glyphosate residues (ppm), and violation in Sheep feed (forage) samples collected from different regions of Baghdad province.

The result of HPLC analysis for all forage samples (Alfalfa, barley, and clover) were above the Maximum Residues Levels MRLs (0.05, 20, and 0.05) respectively, which violated the recommendation of the (EFSA, 2019) [19] for glyphosate (ppm) in Sheep feed.

The highest violation was found in alfalfa, with glyphosate levels over 2,800-fold higher than the MRL, followed by clover at over 2,400-fold the MRL. The lowest violation was detected in barley, where levels were 6.5-fold higher than the MRL.

Glyphosate residues in water samples

The HPLC residues analysis showed that the total positive for the presence of glyphosate residues in(Barn, Field, and Drained)were (2, 3, and 2), with the highest range of glyphosate residues were recorded in Field followed by Barn then Drained, (104.1-106.3), (83.7-85.5) and (0.3-0.5) respectively and highest mean±SE of glyphosate residues were recorded in Field followed by Barn then Drained, (105.20±0.63), (84.60±0.90) and (0.40±0.10) respectively. The result of HPLC analysis for all water samples except Drained water (Barn and Field) were above the Maximum Contamination Level (MCL) (0.7) which violated the recommendation of the (EPA) for glyphosate (ppm) in water. The highest violation was found in Field water, where glyphosate levels were over 150-fold the recommended MCL, while Barn water showed levels over 120-fold the MCL.

Water samples	No. of total samples	No. of positive samples	Means±SE	Violation MCL (0.7 ppm)
Barn	5	2	84.60±0.90	120-fold
Field	5	3	105.20±0.63	150-fold
Drained	5	2	0.40±0.10	< 0.7

Table 2: Level for glyphosate residues (ppm) with violation in Sheep's water samples collected from different regions of Baghdad province

Discussion

HPLC analysis showed that Alfalfa, barley, and clover samples exceeded Maximum Residue Levels. Misuse, not following the instruction label, and glyphosate number and spray rates may have caused these effects. Glyphosate's release led to the commercial success of various formulas, including Roundup®. Also, The first herbicide-resistant crop was genetically modified. Modified genetic herbicide resistance increased glyphosate use, making it the most widely used herbicide. These were "Roundup Ready" (RR) glyphosate-resistant feed crops. Glyphosate also desiccates before harvest. Glyphosate was the second-most-used herbicide on sorghum and barley. Rapeseed, maize, cotton, barley, and beetroots absorb glyphosate. This exposure pathway is significant since roots intercept most glyphosate in agricultural runoff [20].

Large amounts of glyphosate are now being used for control of weeds during the season of growing on fields with genetically modified (GM), glyphosate-tolerant (so-called Roundup Ready®) (RR) crops like (RR barley, RR clover, and RR alfalfa). Additionally, it is now common practice to use glyphosate for the desiccation of crops before harvesting to obtain a harvest-ready crop, , uniformly ripe, and weed-free, for instance, from

(2010 to 2013), (43%) of the wheat samples and (50%) of the analyzed Danish barley samples both contained glyphosate residues [21].

As less expensive Herbicides containing glyphosate entered the market, glyphosate was used as a desiccant before-harvesting. In this use, farmers apply the chemical to crops in the field right before harvest to efficiently kill the plant to ensure it can completely dry out and increase the effectiveness of mechanized harvesting. Chemical desiccation of crops is mostly used in regions of the world with shorter growing seasons and wetter climates, making it more difficult for farmers to guarantee that their crops have enough time to naturally dry before harvest. Despite there are various substances that can perform this task, glyphosate is becoming more prevalent on both (non-GM) and (GM) crops, such as beans, soy, oats, barley and wheat, as a desiccant. Due to the proximity of the before-harvesting spraying to harvesting time, this practice has resulted in increased residues of glyphosate on crops when they are sold, and consequently residues on products of food [22].

Planting glyphosate-tolerant alfalfa allows for the use of glyphosate pesticides for weed control. This could benefit the environment, economy, and agriculture. Such planting strategies would allow alfalfa growers to increase crop safety, fodder quality, and weed control by removing weeds from stands [23,24]. Glyphosate controlled clover and alfalfa weeds without harming clover growth. Studies indicated that glyphosate-controlled clover weeds 90-97%. Clover plants from the first and second seasons had high glyphosate residues (ppm). After 30 days from spraying, clover plants' glyphosate concentrations were lower than at zero time [25]. From the vegetative phase to harvest, glyphosate residues have been identified in plant stems and leaves, associated with application timing. Concentrations were higher when spraying near harvesting, regardless of application rate. Several weed and crop species absorb and translocate 3–38% of the glyphosate sprayed 14 days after spraying. Glyphosate seeps from fields often. Roots can transfer glyphosate, which can affect non-target plants in agricultural canals [17,25]. In week 5 after pre-emergent treatment, glyphosate levels were considerably identified [26]. Glyphosate was found 14 days after treatment [27]. The herbicide may have had too little time to metabolize and dissipate in the plant between sprayings and harvest. AMPA levels changed with use. The timing of application affected grain concentrations. Barley, wheat, and soybeans, which are resistant to glyphosate, [11] discovered herbicide metabolite AMPA and its residues in grains due to metabolism and translocation throughout the plant. As the reproductive stage neared, residual grain levels increased with consecutive treatments. RR Although the package instructions say to apply Alfalfa many times per season, it is often only applied once at the start of the year [22]. The findings of this study were consistent with those of [26], which discovered that higher levels of glyphosate were detectable in crop roots, reflecting the conclusions of [27]. Glyphosate accumulates in varied levels in various plant organs and tissues, as demonstrated by [28]. In a few instances, the levels of glyphosate in sunflower roots and shoots varied greatly. All of these demonstrated that glyphosate could be identified in plants for up to four weeks following treatment and that in soil the glyphosate was absorbed by roots in detectably large concentrations.

However, the World Health Organization [29] found the opposite conclusions of this study. indicating plant organs contain low glyphosate residue. Due to misuse and improper waste disposal, glyphosate can enter aquatic habitats and be present in surface, drinking, and groundwater water, which are used for crop irrigation, animal drinking, field, barn, and drain water.

Spraying glyphosate straight over soil deposits a lot of the chemical. Its interaction with soil elements determines its location regarding plants, the atmosphere, and water [30]. When applied to soil, glyphosate degrades in two stages: the phase of soil solutes, where AMPA, the major metabolite, was formed, and the phase when AMPA and glyphosate adsorb to organic matter and clay [31]. EFSA classifies both compounds as persistent in soils because they break down slowly. More than 1,000 days are needed for 90% of AMPA and glyphosate dissipation [32]. Glyphosate partially disintegrates in a few months in clay-rich soils, while its breakdown product AMPA lasts for years [33].

Despite their adsorption of organic matter particles and clay, colloid movement in macropores dissolves AMPA and glyphosate in groundwater after substantial rain [34]. Wind erosion or runoff can carry AMPA and glyphosate-containing soil particles to surface streams, where they can stay in particulate form, adsorb to bottom sediment, or dissolve in water. There are large amounts of AMPA and glyphosate in sediments and natural streams [35,36]. In addition, falling rain, air, irrigation water, and wastewater plant outputs have shown it [37,38]. AMPA and glyphosate dissolve in many types of water since they are polar compounds. They may discharge into streams and rivers or reach the atmosphere as soil dust that disintegrates in streams and rivers. The half-life of any substance in streams and rivers depends on the bottom sediment composition, pH, and water composition, which can be a "sink," especially if metal ions are present. The median half-life given by [39] is a few to (91) days. US

Geological Survey soil and water tests found AMPA and glyphosate widespread in streams, rivers, drains, ditches, precipitation, soils, and sediments. This study agrees with [41], which indicates South and North American surface waters contain the most [42]. Glyphosate has been found in many water sources in Argentina, with the highest levels (700 µg/L) (0.7 ppm) observed in surface waters [43]. AMPA and glyphosate are often found in drinking water.

However, there was an inconsistency between this study's findings and those for [36]. who found in several European and Asian nations, the quantities of glyphosate in surface and ground- waters were generally modest (<2.5 µg/L).

The consumption of glyphosate has increased over time, and this has raised concerns about the potential herbicide's toxicity as well as its potential effects on the health of humans. Residues glyphosate could reach humans or animals through contaminated water, feed, and food.

Author Contributions

Each author collected, analyzed, and discussed study data. The authors discussed the results and contributed to the final draft.

Conflict of Interest

The authors declare that there is no conflict of interest.

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