Toxicopathological Effects of Thiamethoxam on Hemato-biochemical and Productive Performance of Commercial Laying Hens

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INTRODUCTION

The tremendous increase in the human population along with food security problems has ultimately emphasized extensive agriculture production (Marwan et al., 2019). To enhance the production and preservation of the agriculture products various types of insecticides and pesticides are applied globally (Gul et al., 2019a). Many of these insecticides including organophosphates, carbamates, pyrethroids, and neonicotinoids were introduced in the 19th century and proved their contributions in terms of high yield through pest control (McConville, 2015).

During 2006-07 it was recorded that more than two million tons of pesticides have been used in agricultural farming (Parrón et al., 2014; Korany et al., 2019). Most of the insecticides are the big reason for food and environmental contamination. As the food chain is contaminated so it poses a risk to the public health and other vertebrates (Ghaffar et al., 2015a, 2015b; Ugurlu et al., 2015; Yan et al., 2016; Khater et al., 2018; Naseem et al., 2018). Neonicotinoids are the most important and regularly used among these pesticides and now a day many environmental toxicities of this group have been reported. Thiamethoxam (TMX) and imidacloprid (IDC) have been detected in the surface water frequently so posing a high risk to the avian, mammalian and aquatic biota also (Schaafsma et al., 2015; Paquet-Walsh et al., 2019).

The neonicotinoids are further subdivided into subclasses and one of those is thianicotinyl subclass. The first neonicotinoid is thiamethoxam that belongs to this class. The mechanism of action of TMX includes blocking of nicotinic acetylcholine receptors, resulting in paralysis and eventually death of the insect. So, it has been a very effective compound in controlling the pests like aphids, flea beetles, jassids, wireworms, rice hoppers, whiteflies and thrips (Butcherine et al., 2019). Neonicotinoids have dominated the agrochemical market and around the world, they share about 24 and 80% in the agrochemical and seed treatments, respectively (Maloney et al., 2017).

This study was planned to find out the hemato-biochemical effects of thiamethoxam (TMX) in the commercial layer. For this purpose, a total of 75 birds of 30 weeks age were purchased from a commercial farm. Birds were equally divided into five groups A, B, C, D and E. Standard housing conditions were provided to all birds by providing optimum temperature and humidity. Different dosages of TMX including 250, 500, 750 and 1000 mg/kg.bwt were administered to B, C, D and E, respectively. Group A served as control. The trial continued for 45 days that was the peak production period. All groups were monitored daily for physiological parameters including feed consumption, egg production, and eggshell thickness. Blood with and without anticoagulant was collected for hemato-biochemical parameters. Adverse effects on FCR and egg production were recorded in laying hens. Eggshell thinning was also evident. Anemia was a consistent finding in all the TMX treated groups. Physiological impairments of the liver and kidney biomarkers have also been recorded in treatment groups as compared to control. Thus, it can be concluded here that sub-lethal doses of TMX have adverse effects on production performance, hematology and biochemistry of the laying hens.

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Various crops like barley, canola, sorghum, corn wheat and cotton are sprayed with TMX and imidacloprid to control different pests at different developing stages of these crops (Schaafsma et al., 2016; Paquet-Walsh et al., 2019). Almost all these crops are used as whole grains for human consumption or in the form of meals in poultry feed and set up a toxicity threat in the form of residues and metabolites (Butcherine et al., 2019; Gul et al., 2019b). Previously toxicopathology effects of TMX have been reported in rats, wild birds, broilers, etc., however, such information is scarce in commercial layer hens. Thus, this study was planned to know the toxicopathological effects of sub-lethal doses of TMX on productive performance as well as on the blood and serum biochemistry in commercial layer hens.

MATERIALS AND METHODS

Experimental design: From a commercial farm 75 birds of 30 weeks of age were purchased. Optimal housing conditions were provided to all groups (temperature: 25-37°C; humidity: 60-70%). Drinking water and feed were given ad libitum. This trial continued for 45 days that was peak production time. Birds were divided randomly into 5 equal groups with 15 birds in each group. Group A served as control whereas other groups were treated with different doses of thiamethoxam. Groups B, C, D and group E were treated with the sub-lethal dose of 250, 500, 750 and 1000 mg/kg of body weight, respectively on daily basis as per following the dose calculation by Kumar et al. (2010). The research plan was duly approved by Graduate Studies and Research Board, University of Agriculture, Faisalabad vide letter No. 6025-28 dated: 26 February 2019.

Sample collection: Blood collection was done at 15th, 30th and 45th day of experimental trial. Blood with and without anticoagulant was collected as per following the standard protocols. Feed consumption and egg production were logged on daily basis and egg-shell thickness was recorded at three days interval.

Parameters evaluated

Physiological parameters: Physiological parameters mainly feed consumption, egg production and egg-shell thickness were recorded. Daily feed consumption was measured through the difference between the feed offered and feed consumed, then the average of three days was taken and described as an average feed consumption/day. Egg production was measured through counting daily egg produced by each group and then taking an average of three days for each. Egg-shell thickness was measured every 3rd day throughout the trial period.

Hematological parameters: Blood samples were collected with and without anticoagulant (EDTA). Samples collected with EDTA were used for hematological studies (Ali et al., 2020) while sample without EDTA were subjected to serum extraction and stored at -20°C (Khan et al., 2020) till further use for biochemical studies. Counting of total erythrocytes and total leukocytes was done as per the procedure described by Natt and Herrick (1952). Packed cell volume (PCV) and hemoglobin (Hb) were determined through the procedures described by Sharaf et al. (2013). Erythrocyte indices (MCV, MCH, and MCHC) were also calculated by using the standard formulas. Commercially available kits of M/S Quimica Clinica Aplicada, Spain were used to measure the values of AST (aspartate aminotransferases, catalog # 999500), ALT (alanine aminotransferases, catalog # 990428), TP (total protein, catalog # 997180), Albumin (catalog # 997258), Urea (catalog # 996060) and Creatinine (catalog # 990108). Appropriate standard protocols were followed to estimate the values of all biochemical parameters.

Statistical analysis: The data were analyzed by analysis of variance by using the software Statistix 10. Means of different groups were compared by applying Tukey’s Test.

RESULTS

Feed consumption and production performance: It was observed that the feed consumption was negatively affected by sub-lethal doses of TMX (Table 1). The highest feed consumption was recorded in the control group while the feed consumed at the lowest rate was by group E treated with the highest dose of TMX. So, an inverse relation was recorded with the treatment doses. This trend continued in a linear fashion throughout the trial. Likewise, egg production and eggshell thickness were seen in a linear inverse relationship with TMX as mentioned in Table 1.

Hematological parameters: The remarkable effect was observed on all blood parameters as shown in Table 2. Total erythrocyte count was seen in linear inverse relationship to the dose of TMX. As the dose was increased the number of erythrocytes decreased. The lowermost value of TEC was recorded in group E and at the 45th day of the experimental trial while the highest value was observed in the control group. Likewise, hemoglobin, hematocrit values were also decreased a similar manner to TEC. The erythrocyte indices including MCV and MCH were observed having a direct linear relationship with TMX while MCHC concentration was decreasing with increasing TMX. The leukocytes values also indicated a direct relation in a dose-dependent manner (Table 2). A remarkable increase in eosinophil and basophil was observed in higher treatment groups as compared to those received lower doses of TMX or control group however these were statistically non-significant.

Biochemical parameters: The values of the different biochemical parameters recorded during this trial have been summarized in Table 3 and Fig. 1-2. It has been observed that various markers used for liver and kidney health were affected by the TMX even at sub-lethal doses in laying hens. The enzymes levels (AST and ALT) were significantly increased in TMX treated groups (B-E) as compared to the control group (A) as shown in Fig. 1. Among the treatment groups, the highest levels of AST at 45th day the experimental were observed in group E (350.3±20.03 IU) receiving the highest dose of the TMX followed by D (341.0±14.22 IU), C (324.0±22.33 IU) and B (210.3±18.14 IU), respectively. Similarly, the highest levels of ALT at 45th day the experimental were observed in group E (30.23±18.85 IU) receiving the highest dose of the TMX followed by D (27.7±1.6 IU), C (24.6±2.08 IU) and B (21.4±2.96 IU), respectively. This increase in enzyme values was also statistically significant on 15, 30 and 45th days of the experimental trial (Fig. 1).
Globulin also has a similar pattern like albumin.

Likewise, albumin and globulin were also noticed in a direct inverse relationship. Albumin was not much affected at the start except group E where a significant difference was observed however, as the trial period proceeded, the effect of TMX toxicity became evident in all treatment groups. Among the treatment groups, the lowest levels were observed in group E (3.94±0.21) receiving the highest dose of the TMX followed by D (3.38±0.25), C (4.04±0.41) and B (4.75±0.45), respectively at 30th day of experiment. Further decrease in all the treatment groups was observed at 45th week of trial. Globulin also has a similar pattern like albumin.

A similar trend was recorded in terms of total proteins as shown in Table 3. At the start of the trial a non-significant difference was observed among all the treatment groups except group E. At the 30th day of trial the difference was still non-significant among all the TMX treated groups. Whereas at the 45th day all the treatment groups were statistically different from the control group. The creatinine difference was still non-significant however, as the trial period proceeded, the effect of TMX toxicity became evident among all the treatment groups. Among the treatment groups, the lowest levels were observed in group E (1.73±0.12, 2.3±0.35 and 1.8±0.25 g/dL) levels were significantly higher among group E (the highest dose group) on 15, 30 and 45 experimental days than that of control group A. The creatinine levels among group E were significantly (P<0.05) as compared to that of control birds.

For analysis of kidney damage, the urea and creatinine values were recorded, and values obtained have been summarized in Fig. 2. At the experimental days 15 and 30, urea levels among treatment groups increased significantly (P<0.05) than that of the control group. Whereas on 45th experimental day, urea levels were the highest in group E (64.00±2.64 g/dL) followed by group D (57.00±2.0 g/dL) and group C (54.33±2.51 g/dL) while urea levels did not differ among group B and the control group. The creatinine (1.73±0.12, 2.3±0.35 and 1.8±0.25 g/dL) levels were significantly higher among group E (the highest dose group) on 15, 30 and 45 experimental days than that of other groups (Fig. 2). At the experimental day 30, creatinine levels among group E (2.3±0.35 g/dL and D (1.43±0.27 g/dL) differ significantly than that of control group whereas at the experimental day 45, none of groups showed significant difference among treatment groups except group B that of control group.

Table 1: Effects of sub-lethal doses of TMX treatment on commercial laying hens: feed consumption, egg production and eggshell thickness

| Weeks | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|-------|----------|----------|----------|----------|----------|----------|
| Feed Consumption (g/bird/day) |          |          |          |          |          |          |
| A     | 110.87±1.09a | 107.00±4.00a | 107.02±4.17a | 105.67±4.33a | 104.64±4.39a | 103.91±4.34a |
| B     | 104.74±4.04ab | 97.46±8.70ab | 90.04±12.94b | 85.23±14.03b | 81.94±14.20b | 79.08±14.50b |
| C     | 103.74±3.53ab | 97.16±7.62ab | 88.60±4.01b | 82.75±15.94b | 78.72±16.44b | 75.43±16.75b |
| D     | 100.60±5.29b | 92.00±10.32b | 83.26±15.44b | 78.25±16.05b | 74.56±16.22b | 71.06±16.78b |
| E     | 87.06±5.17c  | 80.13±10.49c  | 66.92±21.35c  | 61.20±20.99c  | 57.20±20.40c  | 53.23±20.68c  |
| Egg production (%) |          |          |          |          |          |          |
| A     | 84.00±5.47a | 83.00±6.74a | 82.00±6.76a | 82.00±6.95a | 81.60±6.87a | 81.66±6.98a |
| B     | 76.00±8.94ab | 74.00±8.69ab | 68.66±1.87ab | 65.00±12.35b | 62.80±12.08b | 60.66±12.29b |
| C     | 72.00±10.95ab | 67.00±9.48bc | 61.33±12.45b | 54.50±17.02b | 49.60±18.36c | 44.66±20.46c |
| D     | 60.00±14.14bc | 52.00±13.16c | 43.33±16.76c | 35.50±20.12c | 30.00±21.12d | 26.00±21.43d |
| E     | 50.00±12.24c  | 35.00±20.68d  | 26.66±21.26d  | 22.00±20.41c  | 18.40±19.72d  | 15.33±19.25d  |
| Egg shell Thickness (mm) |          |          |          |          |          |          |
| A     | 0.35±0.01a | 0.35±0.02a | 0.35±0.02a | 0.36±0.02a | 0.35±0.01a | 0.35±0.02a |
| B     | 0.35±0.02a | 0.34±0.04a | 0.33±0.02ab | 0.34±0.07a | 0.337±0.13a | 0.32±0.08b |
| C     | 0.33±0.12b | 0.32±0.07b | 0.30±0.05b  | 0.29±0.22b  | 0.27±0.16b  | 0.26±0.02c |
| D     | 0.32±0.06c | 0.31±0.13c | 0.30±0.16c  | 0.30±0.08c  | 0.27±0.18b  | 0.25±0.26d |
| E     | 0.31±0.08c | 0.30±0.15c | 0.29±0.13c  | 0.28±0.07c  | 0.25±0.31b  | 0.21±0.07e |

Values in each column under specific parameter bearing different letters differ significantly (P<0.05).

Fig. 1: Values of AST (upper) and ALT (lower) in commercial laying hens in control group (A) and treated with different sub-lethal doses of TMX (group B-E). Bars bearing asterisk on the top differ significantly (P<0.05) as compared to that of control birds.

Fig. 2: Values of urea (upper) and creatinine (lower) in commercial laying hens in control group (A) and treated with different sub-lethal doses of TMX (group B-E). Bars bearing asterisk on the top differ significantly (P<0.05) as compared to that of control birds.
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Table 3: Effects of sub-lethal doses of TMX treatment on hematological parameters in commercial laying hens

| Groups | 15th day | 30th day | 45th day |
|--------|----------|----------|----------|
| Total erythrocyte count (10^6/μL) | | | |
| A | 3.4±0.49a | 3.0±0.20a | 3.9±0.60a |
| B | 2.9±0.06ab | 2.9±0.05a | 2.5±0.15b |
| C | 2.4±0.15bc | 2.2±0.43b | 1.8±0.48c |
| D | 1.9±0.16c | 1.6±0.50bc | 1.4±0.14cd |
| E | 1.8±0.15c | 1.3±0.20c | 1.1±0.05d |
| Hemoglobin (g/dL) | | | |
| A | 9.5±0.91a | 8.3±0.55a | 9.1±0.18a |
| B | 7.5±0.22b | 7.0±0.85ab | 6.8±0.56b |
| C | 6.4±0.37bc | 5.8±0.40bc | 5.8±0.17c |
| D | 5.7±0.17cd | 5.2±1.05cd | 5.0±0.17cd |
| E | 4.8±0.02d | 4.4±0.15d | 4.6±0.11d |
| Hematocrit (%) | | | |
| A | 36.00±2.64a | 34.00±2.64a | 38.00±3.60a |
| B | 34.00±2.64ab | 33.33±0.57a | 30.00±3.00b |
| C | 32.00±3.00abc | 30.33±2.51ab | 30.00±1.73b |
| D | 27.66±2.08bc | 26.66±1.15b | 26.00±2.64b |
| E | 27.33±1.52c | 25.33±1.52b | 23.33±2.30ab |
| Mean corpuscular volume (fL) | | | |
| A | 106.46±7.2c | 113.46±15.3c | 116.56±8.3c |
| B | 115.81±9.3bc | 120.70±21.1c | 131.14±21.1bc |
| C | 131.74±1.0abc | 138.75±32.4abc | 168.71±35.1abc |
| D | 142.31±2.13a | 175.23±63.3ab | 182.20±36.1ab |
| E | 140.22±6.5a | 167.69±5.5a | 239.03±24.7a |
| Mean corpuscular hemoglobin (pg) | | | |
| A | 25.14±5.05b | 27.48±0.36b | 23.17±0.15c |
| B | 26.16±1.22b | 35.33±1.51ab | 37.00±2.00b |
| C | 26.45±3.13b | 38.76±1.66ab | 41.00±3.60b |
| D | 30.85±1.12b | 41.02±3.59a | 41.66±1.32b |
| E | 49.66±10.78a | 34.60±5.40a | 49.00±2.00a |
| Mean corpuscular hemoglobin concentration (g/dL) | | | |
| A | 26.37±1.35a | 24.58±3.37a | 24.10±1.82a |
| B | 22.74±0.41b | 22.23±3.27ab | 20.55±1.21b |
| C | 20.15±0.87b | 19.41±3.23abc | 18.45±0.94bc |
| D | 17.33±1.52c | 17.11±1.92bc | 14.33±0.57c |
| E | 15.33±0.57c | 14.33±1.52c | 13.00±1.00c |
| Total leukocyte count (10^6/L) | | | |
| A | 2.6±0.30a | 2.4±0.40c | 2.7±0.41c |
| B | 2.7±0.20b | 2.9±0.37ab | 3.1±0.10bc |
| C | 3.1±0.11ab | 3.1±0.30bc | 3.4±0.00abc |
| D | 3.2±0.32ab | 3.6±0.25ab | 3.8±0.17ab |
| E | 3.7±0.47a | 4.0±1.11a | 4.1±0.50a |
| Total erythrocyte count (10^6/μL) | | | |
| A | 9.9±0.80a | 8.6±1.23a | 9.9±0.57a |
| B | 8.5±0.52b | 7.7±0.35ab | 5.5±1.25b |
| C | 7.8±0.24b | 6.1±1.26bc | 3.9±1.35b |
| D | 7.5±0.36b | 4.7±0.52c | 3.5±0.80b |
| E | 6.0±0.23c | 4.4±0.63c | 2.9±0.98b |
| Albunin (g/dL) | | | |
| A | 5.5±0.36a | 5.1±0.17a | 7.4±0.47a |
| B | 5.4±0.49a | 4.7±0.45ab | 4.1±0.70b |
| C | 5.1±0.63ab | 4.0±0.41bc | 3.3±0.18b |
| D | 4.8±0.07ab | 3.3±0.25bc | 3.1±0.56b |
| E | 4.1±0.46b | 3.9±0.21c | 2.4±0.14b |
| Globulin (g/dL) | | | |
| A | 4.4±0.15a | 3.4±0.10a | 2.4±0.40a |
| B | 3.1±0.11ab | 2.9±0.16a | 0.9±0.53b |
| C | 2.6±0.55b | 2.0±0.21b | 0.6±0.29a |
| D | 2.6±0.43b | 1.9±0.77ab | 0.3±0.31b |
| E | 1.8±0.61b | 0.9±0.69b | 0.4±0.40b |

DISCUSSION

Among neonicotinoids, thiamethoxam is the most water-soluble insecticide because of its different chemical structure. Its absorption is very fast in plant tissues because of its higher water solubility. Due to their retention in the environment their adverse effects on non-target species have been reported in the literature. Ecosystem integrity has been facing a challenge from neonicotinoids so that’s why their use has been restricted by the European Union (Kurwaldkar et al., 2014; Schaffsma et al., 2016). Still, it is being used in countries like Pakistan to control the pests and applied as sprays on crops or for seed and foliar treatments (Gul et al., 2019a).

The presence of pesticides has been found in the food chain as the residues have been detected in rice polish, bran and rice husks (Telo et al., 2015; Raheel et al., 2019).

In the present study, the feeding behavior of laying hens was dangerously affected by TMX. At the end of the first week there was a non-significant difference among the lower treatment groups and the control group. But as the duration of toxicity was increased significant decrease in feed consumption was recorded. At 4th, 5th and 6th week there was a substantial decrease in feed utilization. These findings have been in accordance with the previously reported effects of pesticides by Sharaf et al. (2010) and Ganguly (2013) in chicken. Reduction in feed consumption has been attributed to the toxic effects of TMX on the liver that impairs the digestive ability of the bird. Eggshell thickness was also affected negatively by TMX. It was seen decreasing on dose- dependent manner except for group B in the first five weeks. At 6th all groups were seen substantially affected by TMX. These conclusions were seen in accordance with the results described by Ganguly (2013). Hemoglobin and PCV values were also decreased in dose dependent manner and it was in accordance with previous reports by Gul et al. (2017) and Sharaf et al. (2010) in broilers and by Qureshi et al. (2016) in fish, respectively. Erythropoietin is a major factor in for erythropoiesis so this decline in TEC, hemoglobin and PCV values can be attributed to the reduction in the synthesis or augmented rate of destruction of RBCs (Fetoui et al., 2008; Kumar et al., 2010; Gul et al., 2017).

Erythrocyte indices including MCV and MCH indicated a direct linear relationship to TMX treatment that results in anemia. Macrocytic hypochromic anemia is due to the increased number of immature erythrocytes;
however, erythrocyte swelling due to hypoxia in individuals treated with toxicants has been defined as a reason for this type of anemia. Toxins cause membrane damage to the RBCs through the production of reactive oxygen species (ROS) (El-Rahman et al., 2019). The TLC has been observed to increase in TMX treated groups. Increased TLC might be the result of immune system response when toxicant is detected and in response to that there is increased production of leukocytes but a continuous exposure for longer duration results in leukopenia resulting in immunosuppression (El-Rahman et al., 2019).

The liver is the most potent target in terms of many environmental toxicants due to its major role in detoxification (Abdel-Daim et al., 2013; Latif et al., 2020). The increased levels of ALT, AST and Urea have a linear relationship with TMX doses in this study and similar trends in the literature have been reported previously (Aslam et al., 2010; Gul et al., 2017). These are indicative of the hepatic damage that ultimately raises their level in the main bloodstream. A direct relationship exists between enzyme levels and hepatic damage (El-Rahman et al., 2019). The higher level of the urea and creatinine has been attributed to the joint excretion by glomeruli filtration and tubular secretions due to the toxic effects on kidneys (Abdel-Daim and Abdeen 2018; El-Rahman et al., 2019; Sharaf et al., 2020).

The relation was inverse in terms of total proteins, albumin, and globulin as the lowest levels were recorded in high dose groups. The possibilities for the reduction in the levels of total proteins, albumin, and globulin have been attributed to the protein breakdown for energy production to counter the stress or that might be due to the stoppage of the synthesis of the proteins (Yonar et al., 2014; Awaad et al., 2019; Abdel-Sattar et al., 2019).

**Conclusions:** Thiamethoxam has adverse effects on the feed consumption and production of the commercial laying hens even at sub-lethal doses level along with a decrease in quality of eggs through egg-shell thickness reduction. It also results in anemia due to toxic effects on RBCs and associated parameters. It also results in the impairment of liver and kidney functions. So, increased enzyme levels (ALT and AST) and urea have been observed. The levels of total proteins, albumin, globulin, and creatinine have been decreased than the control group. Thus, it can be concluded here that sub-lethal doses of TMX have adverse effects on production performance, hematology and biochemistry of the laying hens.

**Authors contribution:** STG, IA, MKS and AK were actively involved in idea conceiving and project designing and execution. AK and MA were involved in data analysis, interpretation and write up of the manuscript. All authors approved the manuscript.

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