Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.052

RESEARCH ARTICLE

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

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ARTICLE HISTORY (20-181)

Received:	April 11, 2020
Revised:	May 08, 2020
Accepted:	May 11, 2020
Published online:	June 08, 2020

Key words:
Commercial Layers
Egg production
Hematology
Thiamethoxam
Toxicity

ABSTRACT

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.

To Cite This Article: Gul ST, Ahamd I, Saleemi MK, Ahmad M, Ahmad L and Khan A, 2020. Toxico-pathological effects of thiamethoxam on hemato-biochemical and productive performance of commercial laying hens. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2020.052

INTRODUCTION

The tremendous increase in the human population along with food security problems has ultimately emphasized extensive agriculture production. 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 organo-phosphates, 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). 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 (Ugurlu *et al.*, 2015; Yan *et al.*, 2016). 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).

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 toxico-pathology 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 toxico-pathological 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: 25-37ºC; 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.

Hemato-biochemical 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 -20ºC (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 Tuckey'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 trail. 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 trail 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 has 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 (350.33±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 (210.33±18.14 IU), respectively. Similarly, the highest levels of ALT at 45th day the experimental were observed in group E (30.23±3.85 IU) receiving the highest dose of the TMX followed by D (27.70±1.60 IU), C (24.66±2.08 IU) and B (21.40±2.96 IU), respectively. This increase in enzyme values was also statistically significant on 15, 30 and 45th days of the experimental trail (Fig. 1).

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

Weeks	1st week	2nd week	3rd week	4th week	5th week	6th week
Feed Consumption (g/bird/day)						
A	110.87±1.09a	107.80±4.00a	107.02±4.17a	105.67±4.33a	104.64±4.39a	103.91±4.34a
B	104.47±4.04ab	97.46±8.70ab	90.04±12.94b	85.23±14.03b	81.94±14.20b	79.08±14.50b
C	103.47±3.53ab	97.16±7.62ab	88.60±14.01b	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	66.93±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±6.99ab	68.66±11.87ab	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±17.00b	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±21.21d	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).

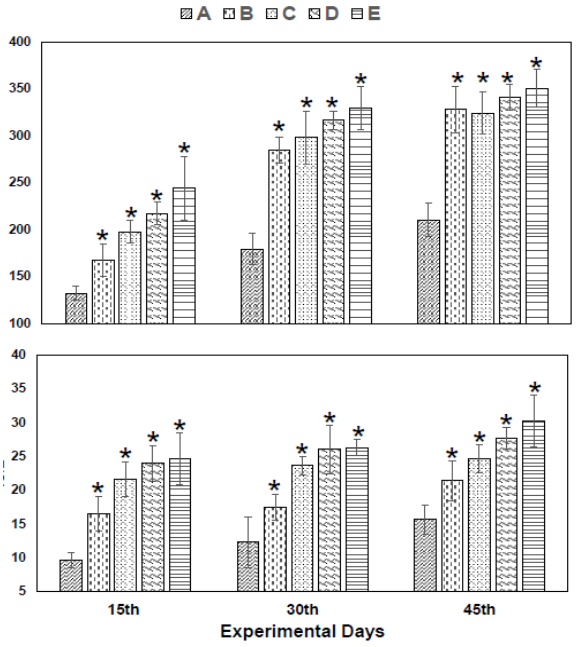

Fig. 1: Values of AST (upper) and ALT (lower) in commercial laying hens in control group (A) and treated with different sub-lethal doses of TMX (group B-E). Bars bearing asterisk on the top differ significantly (P<0.05) as compared to that of control birds.

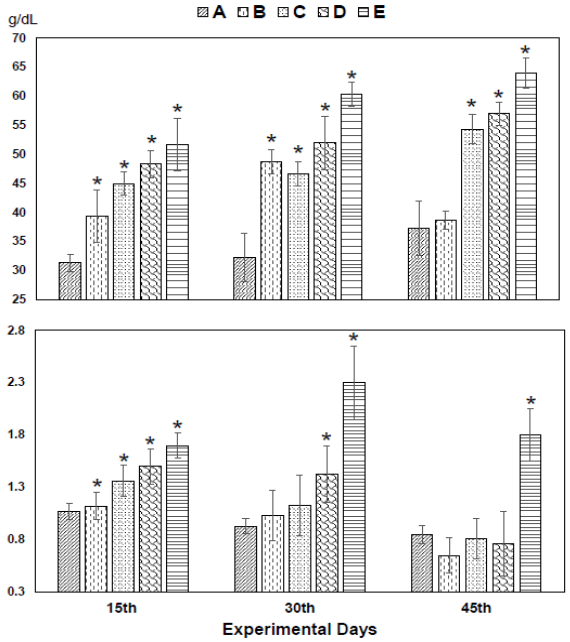

Fig. 2: Values of urea (upper) and creatinine (lower) in commercial laying hens in control group (A) and treated with different sub-lethal doses of TMX (group B-E). Bars bearing asterisk on the top differ significantly (P<0.05) as compared to that of control birds.

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 the TMX treated groups. Whereas at the 45th day all the treatment groups were statistically different from the control group showing the decline in total protein in a linear fashion. 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 have a similar pattern like albumin.

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 (1.7±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 than control group.

Table 2: Effects of sub-lethal doses of TMX treatment on hematological parameters in commercial laying hens

Groups	Experimental Days		
	15th day	30th day	45th day
Total erythrocyte count (10^{12}/L)			
A	3.4±0.49a	3.0±0.20a	3.9±0.06a
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±0.05bc	1.4±0.14cd
E	1.8±0.15c	1.3±0.20c	1.1±0.05d
Hemoglobin (g/dL)			
A	9.50±0.91a	8.30±0.55a	9.11±0.18a
B	7.58±0.22b	7.00±0.85ab	6.68±0.56b
C	6.43±0.37bc	5.80±0.40bc	5.58±0.17c
D	5.70±0.17cd	5.23±0.15cd	5.08±0.17cd
E	4.88±0.02d	4.43±0.15d	4.69±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±2.30b
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±10.1ab	138.75±32.4bc	168.71±35.1abc
D	142.31±12.3a	175.23±8.3ab	182.20±36.1ab
E	144.02±6.5a	189.67±9.5a	233.09±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±1.15ab	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±1.52b
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±3.72ab	20.55±1.21b
C	20.15±0.87b	19.41±2.30abc	18.45±0.94b
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 counts (10^9/L)			
A	2.6±0.30b	2.4±0.40c	2.7±0.41c
B	2.7±0.20b	2.9±0.37bc	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±0.11a	4.1±0.50a

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

Table 3: Effects of sub-lethal doses of thiamethoxam treatment on serum proteins in commercial laying hens

Groups	Experimental Days		
	15th day	30th day	45th day
Total protein (g/dL)			
A	9.9±0.80a	8.61±1.23a	9.90±0.57a
B	8.5±0.52b	7.73±0.35ab	5.56±1.25b
C	7.83±0.24 b	6.14±1.26bc	3.91±1.35b
D	7.51±0.36b	4.77±0.52c	3.51±0.80b
E	6.04±0.23c	4.43±0.63c	2.90±0.98b
Albumin (g/dL)			
A	5.55±0.36a	5.13±0.17a	7.41±0.47a
B	5.41±0.49a	4.75±0.45ab	4.61±0.78b
C	5.15±0.63ab	4.04±0.41bc	3.30±1.18b
D	4.83±0.07ab	3.38±0.25bc	3.17±0.56b
E	4.15±0.46b	3.94±0.21c	2.45±1.34b
Globulin (g/dL)			
A	4.40±1.05a	3.48±1.10a	2.48±0.40a
B	3.12±1.01ab	2.98±0.16a	0.94±0.55b
C	2.68±0.55ab	2.09±1.21ab	0.61±0.29b
D	2.67±0.43ab	1.39±0.77ab	0.34±0.31b
E	1.89±0.61b	0.49±0.69b	0.44±0.40b

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

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 (Kurwadkar *et al.*, 2014; Schaafsma *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).

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 due 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 effected by TMX. These conclusions were seen in accordance with the results described by Ganguly (2013). As the pesticides and chemical residues are associated with altered hormonal and neuro-endocrinal functions due to the activation of the stress mechanisms in many species. These chemicals also have a significant effect on gonadal steroid hormones resulting in delayed sexual maturity, egg-shell thinning, embryo mortality and lower production (Bouwman *et al*., 2019).

A substantial decline in TEC was measured with the dose of TMX. The overall effect of TMX was seen negative on TEC of experimental birds. These results were found in line with presented by Gul *et al.* (2017), Sharaf *et al*. (2010) in chicken. Lower no of TEC in TMX treated birds could be due to the reduced production of RBCs due to defective bone marrow (Khan *et al.*, 2009). Hemoglobin and PCV values were also decreased in dose dependant 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).

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, LA and MA were involved in data analysis, interpretation and write up of the manuscript. All authors approved the manuscript.

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