In-Ovo effect of Ochratoxin A and its amelioration with L-tryptophan in White Pekin duck egg

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By

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CERTIFICATE-I

This is to certify that the thesis entitled **"In-Ovo effect of Ochratoxin A and its amelioration with L-tryptophan in White Pekin duck egg"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Veterinary Clinical Medicine, Ethics and Jurisprudence)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Rajeev Kumar** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

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ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"In-Ovo effect of Ochratoxin A and its amelioration with L-tryptophan in White Pekin duck egg"** submitted by **Rajeev Kumar** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Veterinary Clinical Medicine, Ethics and Jurisprudence)** has been approved/ disapproved by the students' advisory committee and the external examiner.

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LIST OF ABBREVIATIONS

%	:	Percent
µmol	:	Micromole
AFs	:	Aflatoxins
ALT	:	Alanine aminotransferase
ANOVA	:	Analysis of variance
AST	:	Aspartate aminiotransferase
BUN	:	Blood urea nitrogen
DC	:	Differentiated cells
DLC	:	Differential leukocyte count
DNA	:	Deoxyribonucleic acid
DPX	:	Dibutylphthalate polysterene xylene
EDTA	:	Ethelene Di amine Tetra acetic acid
et al.	:	*et alii*
g	:	Gram
G	:	Group
H&E	:	Hematoxylin and eosin
i.e.	:	that is
Ig	:	Immunoglobulin
IU	:	International unit
M	:	Molar
mg	:	Milligram
min	:	Minute
ml	:	Milliliter
mM	:	Millimolar
N	:	Normal

NaB	:	Sodium bentonite
NaHCO3	:	Sodium bicarbonate
ng	:	Nanogram
nm	:	Nanometer
nmol	:	nanomole
NSS	:	Normal saline solution
OTA	:	Ochratoxin
PBS	:	Phosphate buffered saline
PCV	:	Packed cell volume
PMBC	:	Peripheral blood mononuclear cells
PVPP	:	Polyvinylpolypyrrolidone
RBC	:	Red blood cell
sec	:	Second
SGOT	:	Serum glutamate oxaloacetate transaminase
SGPT	:	Serum glutamate pyruvate transaminase
TEC	:	Total erythrocyte count
TLC	:	Total leukocyte count
Trp	:	L-Tryptophan
U	:	Unit
U/L	:	Unit per Liter
UC	:	Undifferentiated cells
viz.	*:*	Videlicet (namely)
WBC	:	White blood cell
µg	:	Microgram

ABSTRACT

Productivity of ducks is extensively affected by mycotoxins like Ochratoxin-A (OTA) and aflatoxins (AFs) and hence reducing the economy of poultry farmers. The effect of Ochratoxin-A is also seen in ducklings hatched from Ochratoxin-A consumed ducks. Residual effect is also detected in eggs and meat of poultry birds taking Ochratoxin-A contaminated feed. The present study was conducted to evaluate the deleterious effect of Ochratoxin-A and its amelioration by L-Tryptophan in terms of change in haematobiochemical, histopathological and production parameters in ducklings which were produced from Ochratoxin-A inoculated eggs. For this purpose 200 eggs of White Pekin ducks were divided into four groups (G-1, G-2, G-3, G-4). Group 1 was control, Group 2 was inoculated with L-Tryptophan (Trp) @5 mg/egg on 22nd day of incubation. Group 3 was injected with OTA (50 ng/egg) on 0 day of incubation. Group 4 was injected with both Ochratoxin-A and Tryptophan. Hatched chicks were maintained in brooding sheds under standard scientific conditions. Body weight of birds were recorded weekly up to 4 weeks of age to study the effect of Ochratoxin-A on growth. After 28 days blood was taken from 6 ducklings of every group to examine hematobiochemical parameters. Organs like liver, kidney, bursa and thymus were collected from dead embryos of unhatched eggs. There was difference in body weight was noticed between all four group from day 0 to 4th week and Tryptophan (G-2) showed maximum growth while toxin treated group (G-3) showed least growth. There was significant decrease in the level of Hb, TLC, PCV and TLC count in in OTA treated group in comparison to control group whereas, no significant difference was observed in lymphocyte, heterophil, basophil and eosinophil. However, all haematological parameters were significantly ameliorated in OTA+Trp treated (G-4) birds. A substantial increase was evident in the values of BUN, Creatinine, Uric acid, SGOT (AST) and SGPT (ALT) in comparison to control which were significantly decreased in OTA+Trp (G-4) treated birds as compared to Ochratoxin-A treated (G-3) birds. There was significant decrease in total protein and globulin in Ochratoxin-A treated birds as compared to control group Whereas, there was no significant difference is seen in values of glucose, triglyceride, cholesterol and albumin. A characterstic pathomorphological changes were observed in liver, kidney, thymus and bursa in Ochratoxin-A inoculated eggs. Vacuolar degenerations, dissolution of hepatocytes along with infiltrations of inflammatory cells and congestion were evident in liver parenchyma while narrowing of tubular lumen, degeneration to necrosis of renal tubular epithelial cells were prominent findings in kidney through histopathology. In majority of cases, atrophy of thymus with depletion of lymphoid follicles also noticed. So it can be concluded that in ovo feeding of L-tryptophan stimulates growth, improves blood haematological and serobiochemical profile as compared to Ochratoxin-A infected or control group as well as it also overcomes the harmful effects of Ochratoxin-A on growth, performance and various body parameters.

INTRODUCTION

In India, the poultry business is expanding tremendously and making a significant contribution to the agricultural economy. India ranks third globally in the production of eggs and eighth globally in the production of ducks. In the poultry sector, duck production is second only to chicken production. India's highest duck population is found in West Bengal, which is preceded by Assam, Kerala, AP, TN, UP, Bihar, and Odisha. Ducks produce about 20 more eggs than backyard chicken, and their eggs are also 10-15 grams larger. They also grow much more quickly than chickens. A chicken's egg only makes up 3.3% of a chicken's body weight as compared to a duck's egg, which accounts for 4.5% of a duck's weight. Duck meat has a good nutritional value because it contains a range of polyunsaturated fatty acids (PUFA) and an appropriate ratio of omega-6 to omega-3 fatty acids.

In tribal areas of Odisha, raising ducks in village community ponds improved everyone's standard of living. They used small, ineffective ponds that were not in use to raise ducks. Ducks are waterfowl, and they share genetic ancestry with geese and swans.

The upper limit for OTA in poultry feed is 0.1 mg/kg, which was set by the European Commission (EC, 2006). A general maximum OTA limit of 5 µg/kg in cereals and 3 µg/kg in cereal products was proposed by the World Health Organization.

When taken in sufficient quantities, mycotoxins-secondary metabolites of toxico-genic fungus species found in animal feed and food-cause negative biological consequences. Numerous common genera and species of fungi are known to produce toxins under favourable conditions of temperature (24–250°C), moisture (15%), and 90–95% relative humidity.

Beyond simply causing death, fungal poisons have a profound effect on animal health. In comparison to immediate morbidity and lethality, the economic effect of decreased production, reduced feed efficacy, decreased weight gain, decreased meat and egg production, increased disease incidence, damage to multiple vital organs, and interference with reproduction is significantly higher (CAST, 2003).

Ochratoxins are the collection of secondary metabolites with similar structures that are formed by a number of toxigenic *Aspergillus* and *Penicillium* species. They have a various harmful effects on both people and animals and contaminate a number of raw agricultural products.

The formation of reactive oxygen species (ROS), which inhibits protein synthesis, lipid peroxidation, and disruption of genetic materials like DNA as a result of OTA-mediated oxidative damage, is the most significant harmful effect of OTA in animal cells (Ringot *et al*., 2006).

Evidence has been gathered and reported that suggests oxidative damage plays a part in the toxicological features of OTA that include changes in genetic materials, deformations, embryo toxicity, hepatotoxicity, and nephrotoxicity (Hassan *et al*., 2012).

There are various toxicities that define OTA. The effects of this substance on several avian and mammalian species, including nephrotoxicity, hepatotoxicity, immunotoxicity, teratogenicity, genotoxicity, mutagenicity, and neurotoxicity, have been demonstrated in numerous studies (Kumar *et al*., 2004; Hassan *et al*., 2010, 2011).

After laying hens were fed OTA-contaminated feed with 0.5 or 5 mg for 14 days, OTA was found in all bodily tissues, with the kidney having the highest amount (124 ppb) (Frye & Chu, 1977).

In chickens, OTA's effects on teratogenicity and embryotoxicity have been demonstrated both on its own and in conjunction with other mycotoxins, reviewed by (Wangikar *et al*. 2007, 2005; Wangikar, Dwivedi, and Sinha 2004; Wei and Sulik 1996; Vesala, Vesely, and Jelinek 1983; Gilani, Bancroft and Reily, 1978).

By producing anti-inflammatory cytokines, tryptophan affects intestinal epithelial cells and improves immune system protection by raising blood immunoglobulin levels. For chicken, *in ovo* feeding is essential for enhancing growth performance, feed conversion ratio, intestinal health, and immunity.

Tryptophan is a necessary amino acid for poultry, and when added to diets in amounts that are below but not above the recommended levels, it can linearly improve Pekin ducks' growth performance (Adeola, 1998).

Tryptophan, a potential nutrient, has a significant effect through *in ovo* feeding, which improves the chicks' body weight growth, immunity, and overall performance. (Nayak *et al*., 2018).

According to a recent study, therapy with the tryptophan precursor 5-hydroxytryptophan (5-HTP) raised the levels of IgA in chickens' ileum and duodenum.

Therefore, the goal of the current study was to ascertain how *in-ovo* injection of OTA affects the liver and kidneys as well as the ameliorative effects of L-Tryptophan on OTA infected eggs.

Objectives of study

1. To study the effect of OTA induced toxicity in duck eggs.
2. To study gross and histopathological changes of various organs.
3. To study the effects of ochratoxin A induced toxicity on haematological and serum biochemical parameters.
4. To study the ameliorative effect of L-tryptophan in OTA induced White Pekin duck eggs.

REVIEW OF LITERATURE

2.1 Ochratoxin

Mycotoxins, the harmful secondary metabolites of fungi produced mostly by numerous species of Aspergillus, Fusarium, and Penicillium, have been known to impair animal and human health for over a thousand years, but little is known about how these complicated chemicals affect chicken, which is often quite sensitive. About 200 different fungal species are known to create mycotoxins. Aspergillus, Penicillium and Fusarium are the three genera that comprise the majority of the fungi that produce mycotoxin.

Larsen *et al.* (2001) told that *Penicillium verrucosum* and *Penicillium nordicum*, two *Penicillium* species, both have the capacity to generate OTA.

Scott *et al.* (1978) observed that OTA is frequently found naturally in foods and feeds of both plant and animal origin. OTA accumulates in the food chain because of its extended half-life and poses a concern to both human and animal health due to its severe toxicity, ubiquitous incidence, and wide range of commodities that it can contaminate.

Peckham *et al*. (1971) told that due to OTA's negative impacts on performance and health, the poultry sector suffers enormous losses. It reduces body growth and decreases feed consumption, results in worse feed conversion, and raises mortality, reviewed by (Huff *et al.*, 1974 and Verma *et al*., 2004).

Zhai *et al.* (2021) stated that poultry exhibit a higher sensitivity to OTA toxicity than mammals do.

Piskorska-Pliszczyka and Juszkiewicz (1990) observed the presence of the toxic substances and its compounds in poultry eggs may also be related to consumption of OTA by bird.

Van Egmond (1994) stated that several countries established rules for maximum permissible levels of mycotoxins in feed after findings of international investigations revealed evidence of significantly widespread contamination of OTA in poultry feeds at farms.

Galtier *et al*. (1981) observed that pigs absorb the poison more quickly than chickens, but pigs have a far higher oral bioavailability (about 60%) than chickens (approximately 40%).

Chang *et al*. (1979) stated that Ochratoxin A is a very poisonous member of the mycotoxin family, having the lowest effect on bird growth and the median lethal dose.

Huff *et al.* (1974) studied that for Japanese quail and turkeys, the oral median fatal dose had been seen to be approximately 5.9 and 16.5 mg/kg body weight (BW) and 0.5 and 2 to 4 mg/kg body weight, respectively.

Hoehler and Marquardt (1996) animals affected with harmful actions of OTA have been treated nutritionally in a variety of ways, such as by using particular adsorbents to prevent mycotoxin in the digestive fluids and antioxidant chemicals.

To lessen the negative effects of OTA, animal feeds have included esterified glucomannan (Raju and Devegowda, 2000), activated charcoal, bentonite and cholestyramine (Bauer, 1994), and hydrated sodium calcium aluminosilicate (Huff *et al*., 1992; Santin *et al*., 2002).

According to Burditt *et al.* (1984) different species of chicken would react differently to ochratoxicosis.

In an analysis, Marquardt and Frohlich (1996) first and foremost reported outbreak of ochratoxicosis in broiler, laying and turkey birds in fields. High mortality, kidney toxicity, and reduced intake of food were the main adverse consequences seen in turkeys. Nephropathy and air sacculitis instances, as well as a decline in body growth and feed effectiveness, were seen in broiler chicks. Nephropathy was observed in laying hens, combined with a decline in egg production and quality.

Giambrone *et al.* (1985) observed that mycotoxicosis is typically brought on by ingesting small amounts of fungal metabolites, and it manifests as non-specific modifications including immunosuppression and subcutaneous haemorrhage as well as a demonstrable decline in farm productivity performance.

2.2 Mechanism of toxicosis

OTA's exact mode of operation is unknown. Some writers, however, have hypothesised that OTA might prevent the production of enzymes and other proteins by competitively blocking phenylalanine-tRNA (Ueno, 1991; Council for Agricultural Science and Technology, 2003).

Pattison *et al.* (2008) told that ochratoxin inhibits phenylalanine-tRNA by competitive inhibition, which prevents the creation of enzymes and other proteins.

Betina (1989) studied that OTA primarily affects the proximal renal tubules, where it inhibits the lipid peroxidant enzyme phosphoenolpyruvate carboxylase and changes the structural and functional renal capacity to metabolise calcium.

According to Pitt (2000), the morphology of the kidney's proximal tubules shows degeneration and fibrosis. Functionally, both tube and the kidney's ability to concentrate urine are compromised.

Depending on the chemical makeup of the mycotoxins, the liver, kidneys, or alimentary canal of the fowl will process them. Major public health concerns result from their transfer to poultry products like meat and eggs, which has unfavourable impacts on human health. The GIT mediates mycotoxin absorption and subsequent tissue distribution.

Fukui *et al.* (1987) told that Ochratoxin A has been categorised by the International Agency for Research on Cancer (1993) as a potentially carcinogenic agent (category 2B) to humans notwithstanding reports that it may have teratogenic and genotoxic effects.

2.3 Sources of OTA contamination

Gumus *et al.* (2018) found that 72% of samples of feed used for laying hens had OTA, the bulk of which contained ochratoxin @ 5 to 19 mg/kg.

Magnoli *et al*. (2007) observed that extra, 25 to 30 mg/kg of OTA was found in bird feed from various South American nations. OTA exposure in chickens is primarily caused by contaminated feed. However it is well recognised that contaminated cereal grain straw poses a similar or even higher threat to avian health, bedding studies have received much less attention.

According to Refai *et al*. (1996) *A. ochraceus* and *P. verrucosum* produces ochratoxin frequently (60%) in straw and at an unexpectedly high level (8,000 mg/kg). Additionally, dried alfalfa plants and hay, which are sometimes added as the bedding material, contained OTA.

Iqbal *et al.* (2014) told that additionally, the tissues and eggs of the bird may contain OTA toxin. According to reports, OTA was found in meat samples (41% positive samples), where the mean level was 1.41 mg/kg, and eggs (35%), where the average level was 1.17 mg/kg.

According to Hassan *et al*. (2012) after 21 days of feeding with 5 mg/kg of OTA-contaminated feed, the ochratoxin concentration in eggs attained its peak (7.4 mg/kg).

Niemiec *et al*. (1994) also proved that OTA were found in adult bird eggs, blood , hepatic and renal tissues, as well as day-old chicks hatching from eggs placed by birds which are fed tainted feed.

2.4 Toxic Effects of Ochratoxin A

Liuzzi *et al*. (2016) told that the most prevalent and harmful ochratoxin is called Ochratoxin A.

It has been demonstrated that it is immunotoxic, teratogenic, nephrotoxic, and hepatotoxic to chicken (Hassan *et al* 2012).

Saleemi *et al*. (2009) stated that India has been documented to include ochratoxin A (OTA), a mycotoxin recognised for its nephrotoxic effects, in finished chicken feed and its constituent parts. This statement is also supported by Rehman, Bhatti, and Hanif (2003).

Burns and Dwivedi (1986) told that similar to other species, the primary target organ of ochratoxicosis in poultry appears to be the kidney, while it can also affect the liver, gastrointestinal tract, lymphoid organs, skeletal system, haematopoietic tissues, and reproductive systems.

Zahoor-ul-Hassan *et al.* (2010) reported that Ochratoxin has been classified by the International Agency for Research on Cancer (IARC; 1993) as a possible human carcinogen in addition to having hepatotoxic, immunosup (2B). Same statement was also supported by Wangikar *et al*. 2007.

Wangikar *et al.* (2007) reported the OTA's teratogenic and embryotoxic effects in rat, rabbit, and chicken, both on its own and in conjunction with other mycotoxins.

Carlson and Semeniuk *et al*. (2012) reported on a great decline in the number of ig bearing cells in the spleen and bursa of Fabricius as well as poor production performance of the offspring of breeder hens kept on OTA-mixed ration (1971).

Vander Merwe *et al.* (1965) found that a deadly oral dose of ochratoxin would be comparable to that seen for aflatoxin B1 if it caused the death of 50% of the ducklings receiving it.

Choudhury and Carlson (1971) observed that Ochratoxin was shown to be more hazardous to chickens than aflatoxin and embryonic sensitivity decreases with the increasing age of the embryo.

According to data analysis by O'Brien *et al*. (2010) the OTA molecule can have a variety of adverse effects on numerous species of animals, including renal damage, liver toxicity, neurotoxic effects, teratogenic effects, and immunotoxicity. It can also cause kidney and liver cancers in mice and rats. However, the toxicity of the OTA molecule varies depending on the sex, species, and cellular type of the tested animals.

Pfohl-Leszkowicz *et al*. (2007) demonstrated that giving OTA to rats during their gestation caused numerous CNS abnormalities.

Soleas *et al.* (2001) observed that Ochratoxin can be seen of as a potential reason of certain lesions and harm at the brain level, as a result, it appears that this material is very poisonous to nerve cells and capable of reaching the neural tissue at any time (brain, retina).

Based on a study by Brown *et al.* (1976) OTA is a powerful teratogen for lab animals. It can accumulate in embryonic cells and cross the placenta, causing various morphological malformations.

Stoev *et al*. (2021) observed that chicks given the feed contaminated with Ochratoxin at a dose of 5 mg/kg for 6 and 10 weeks developed macroscopical, histopathological, haematological, and biochemical alterations. The hematopoiesis of chicks was affected, as indicated by a mild anaemia in their serum concentrations. Furthermore, elevated serum levels of urea, creatinine, and uric acid were detected, as well as lower serum total protein levels and high urine protein excretion, all of which point to potentially severe kidney damage. Any one of these metabolic problems may contribute to the liver and kidneys excessive weight as these two organs engaged in the detoxing and removal of OTA from blood. On the other hand, due to the reduction in antibody-producing cells, certain lymphoid organs, such as the thymus, spleen and bursa of Fabricius, showed degenerations and reduced weight.

Santin *et al.* (2002) discovered internal organ alterations in chickens fed a diet containing OTA at a rate of 2 mg/kg for 3 and four 4 weeks, comprising enlarged, yellowish, and friable upon palpation livers and pale, dilated kidneys. More evidence points to OTA's potential involvement as a genotoxic carcinogen as it leads to the formation of oxidative DNA lesions and the formation of a covalent bond between the damaged DNA and OTA reactive metabolites. Animal health effects of OTA are severely affected by its kinetic (i.e., toxicokinetic and toxicodinamic) properties, which vary between ruminant and non ruminants. In monogastric animals (such as swine and fowl species), the stomach and intestines adsorbs the ochratoxin with no pre-degradation or very little pre-degradation.

As per to O'Brien and Dietrich (2005) ochratoxin has been linked to a broad range of toxicological effects in both people and animals, comprising renal toxicity, genotoxicity, teratogenic effects, neurodegeneration, and immunotoxicity.

According to Hassan *et al.* (2012) the rise in OTA in meal for reproductive hens decreased the amount of fertilised eggs' hatchability due to an increase in embryo mortality at different stages of development. OTA shows the worst embryotoxicity of all known mycotoxins. Age of the birds is also critical because it is thought that young birds are more sensitive to OTA than older birds are (Adetunji *et al.,* 2017).

According to Ribelin and Fukushima (1978) renal impairment is the major harmful outcome of OTA. All non-ruminant mammals show the potential renal toxicity of this toxin. There have been indications of OTA's toxic effects on poultry.

2.5 Effect of OTA on growth parameter

Rached *et al*. (2008) OTA has been proven in numerous studies to have harmful effects on mammals, including diarrhoea, haemorrhage, immunosuppression, and growth retardation. Broiler chicks, both male and female, were given OTA for eight weeks. All groups receiving OTA saw a reduction in the effects of continuous feeding of graded doses of ochratoxin body weight gain (0.5, 1.0, 2.0 ppm).

Prior *et al.* (1980) observed that males experienced depression more severely and for a longer period of time than females, and it was related to the amount of OTA exposure.

Xin Liu *et al.* (2020) found that when the inclusion of OTA increased to 1209 mg/kg of feed, the feed efficiency, % weight gain, final body mass and specific growth rate decreased ($p < 0.05$). Feed intake (FI), intestinal length index (ILI), and intestinal weight (IW) decreased when the amount of OTA in meal was increased to 1612 g/kg of diet (p 0.05). Furthermore, as ochratoxin concentrations increased, the concentration of residual OTA in three intestinal segments also elevated steadily.

Dwivedi and Burns (1986) showed decreased growth rate after feeding larger concentrations of OTA, in comparison to Micco *et al*. (1988) and Prior *et al*. (1980), who discovered no weight depression after feeding 1 ppm OTA, and with numerous other toxicity trials where Ochratoxin was noticed to depress rate of growth in a dose-dependent fashion, with a least growth inhibitory level of 2 ppm.

Huff and Ruff (1982) investigated that feeding OTA-contaminated diets to birds may affect their secondary infections and increase their chances to toxicity. An accelerated decrease in body weight and an increase in feed conversion ratio have been seen in chicks fed OA and contaminated with *Eimeria acervulina* or *Eimeria tenella*..

In diet that was infected with OTA (2 mg/kg), Raju and Devegowda (2000) discovered that adding AFs B1 (3 mg/kg) or T2 (3 mg/kg) had a detrimental additive effect on the body weight gain of broilers. Study examined at how Ochratoxin (1, 2 and 4 mg/kg), AFs B1 (0.5, 1 and 2 mg/kg), and their mixture, harmed laying chickens (Verma *et al*., 2003). The co-contamination adversely affected the body's ability to store protein, the amount of energy needed for maintenance, and the quantity of energy stored in the body and eggs.

Jelinek *et al.* (1989) stated that mycotoxicosis has a negative impact on the poultry business by slowing growth and lowering feed conversion and reproductive efficiency.

2.6 Effect of OTA on haematology

Corrier (1991) when OTA is consumed by birds, it damages their hematopoietic system and suppresses hematopoiesis. In addition, it has immunosuppressive actions on the thymus, bursa of Fabricius, spleen, and lymph nodes (reviewed by Bennett, 2003), inhibits the synthesis of blood cells, encourages the decline of haemoglobin, and causes leukopenia and lymphopenia to manifest in broilers. Such effects were seen by Dwivedi and Burns (1984) at concentrations greater than 2 ppm, however they were more pronounced at 4ppm.

As per Maxwell and Robertson (1998) after OTA exposure, an alteration in the circulation of heterophils was observed. Heterophils are the second most prevalent type of leukocyte in the peripheral circulation of the majority of avian.

Bharathi *et al.* (2014) reported that A significant ($P<0.05$) decrease in the PCV was observed in the toxin fed group in the 4th week and in TEC in the 4th week and A significant ($P<0.05$) decrease in the MCV in the 4th week and MCH in the 4th week. A significant ($P<0.05$) increase in the MCHC was observed in the 4th week in the OTA toxin fed group.

In birds, anaemia is manifested by alowered haemoglobin content and a reduction in the quantity of red blood cells (Huff *et al*., 1988). Haematopoiesis is reduced by OTA, which damages the haematopoietic system.

Corrier (1991) told that additionally, OTA has immunosuppressive effects on the lymph node, spleen, thymus, bursa of Fabricius, and myelotoxicity, reducing the synthesis of blood cells, fostering a drop in haemoglobin, and causing leukopenia and lymphopenia to occur in broilers (reviewed by Bennet, 2003).

Denli *et al*. (2008) observed that remains of OTA have been detected in the blood, tissues, and eggs of chickens fed ochratoxin contaminated feed in laboratory experiments.

Kozaczynski (1994) discovered a rare event in birds: enlargement of the capillary walls of the nephrons. Other investigators have also seen a decrease in lymphocytes in the pulpar regions of the spleen.

Dwivedi and Burns, (1984) discovered a remarkable degree of lymphocyte depletion, germinal centres, and sometimes it was it was difficult to distinguish between red and white pulp.

2.7 Effect of OTA on biochemical parameters

According to Stoev *et al*. (2002) excessive urine protein excretion and elevated blood concentrations of uric acid, urea and creatinine, as well as decreased concentratioins of serum total protein, suggest that the kidneys may have been severely damaged. Histopathological analyses revealed hyperplasia of the biliary epithelium, vacuolation and megalocytosis of hepatocytes and hypertrophy of kidneys proximal tubular epithelial cells.

Indresh *et al*. (2013) told that uric acid was noticed in the group fed OTA plus T-2, and the maximum fall of serum total significantly ($P<0.05$) reduced in the groups protein, albumin, and highest raised levels of serum receiving OTA or T-2 toxin alone or in addition with any other substance.

Tansakul *et al.* (2012) observed that the quick rise in AST and CK levels in the treatment groups following the initial (2-week) exposure period. These alterations could be linked to early-stage renal tubule and hepatic cell damage (evident microscopically). Uric acid is a by product of the metabolism of amino acids and is predominantly eliminated as a dry mass through the renal tubules. Due to kidney disease, serum uric acid may be increased because of the reduced excretion of the substance.

Kalorey *et al*. (2005) found that significant reductions in serum protein levels were caused by ochratoxin A at 2 and 4 ppm levels. At the 21st and 42nd days, broiler chicks given Aflatoxins (0.2 ppm) and OTA (0.2 ppm), either alone or in combination, showed a variable decrease in serum total protein and an increase in serum creatinine and uric acid.

Sawarkar *et al.* (2011) when exposed to a combination of toxins (Aflatoxin 0.5 ppm + Ochratoxin 1 ppm and aflatoxin 1 ppm + ochratoxin 2 ppm), broilers' blood protein levels were considerably lower than in the control group. In line with this observation, broiler chicks fed a combination of 0.1 ppm aflatoxin and 0.1 ppm ochratoxin had considerably lower blood total protein levels.

Elaroussi *et al.* (2008) found that in birds receiving 2 ppm AF, there was a noticeable rise in bilirubin levels. Broilers fed 2 ppm AF, a combination of 1ppm AF with 2ppm OA, and 2ppm AF with 4 ppm OA all showobserved that broilers were shown to have elevated serum uric acid levels as a result of being fed OTA at a concentration of 0.4 to 0.8 ppm.

As per Elaroussi *et al.* (2008) serum creatinine concentration was markedly increased by ochratoxin at a concentration of 4 ppm. This discovery supported earlier results using OTA at 0.4-0.8 ppm (reviewed by Stoev *et al*., 2000). Significant increases in SGPT, SGOT, and creatinine were seen in chicken fed with a high amount of OTA contamination.

Huff *et al.* (1988) sensitive markers of ochratoxicosis include changes in the serum levels of a number of amino acids, proteins their metabolites and the action of a number of enzymes.

Marquardt and Frohlich (1992) observed that levels of total protein, albumin, globulin, potassium, cholesterol and triglycerides as well as increased levels of uric acid, creatine, and serum ALP and GGT activity are among the biochemical symptoms of ochratoxicosis.

Kalorey *et al.* (2005) in fact, the drops in blood triglyceride and cholesterol levels during ochratoxicosis may be evidence of decreased liver metabolism.

Huff *et al*. (1992) told that Chickens exposed to OTA also showed similar increases in relative liver weights while chickens fed OTA-contaminated diets showed an increase in serum uric acid content (Hoehler and Marquardt, 1996).

Serum phosphorus levels were dramatically lowered after feeding meals contaminated with OTA. Gupta *et al*. (2005) earlier reported similar findings and Huff *et al*. (1980) found that feeding broiler chicks 2 mg/kg of OTA lowered bone breaking strength. The decrease in phosphorus concentration has been linked to kidney impairment.

Additionally, it results in a decrease in total blood proteins (Huff *et al*., 1988; Stoev *et al*., 2000) a suppression of immunological function (Chang *et al*., 1979; Dwivedi and Burns, 1984; Stoev *et al*., 2000, 2002; Santin *et al*., 2002; Politis *et al*., 2005), and a reduction in blood coagulation (Raju and Devegowda, 2000).

2.8 Effect of OTA on histopathological parameters

Ram Singh *et al*. (2019) found that in histological analysis of the renal sections of broiler birds fed contaminated feed with 200 ppb OTA revealed considerable degeneration of the renal parenchyma's cellular organisation and architecture, cloudy swelling, and a great number of irregular tubules (atropic changes in the tubule) with necrosed and degenerated epithelium, dilated lumens, and multifocal aggregates of mononuclearcells. Birds given OTA (200 ppb) displayed extensive degenerative alterations in their hepatic cells, comprising vacuolated hepatocytes, dilated and congested sinusoids, hyperplastic bile ducts, and multifocal clusters of mononuclear cells surrounding the bile duct and portal area.Tansakul *et al*. (2012) observed that Kidney damage from OTA includes epithelial cell necrosis and mild to moderate degradation in the renal tubules. The therapy group's liver lesions seemed to have modest fatty degeneration and coagulative necrosis of hepatocytes. No spleen lesions were visible.

Stoev *et al*. (2000) found that weights of the lymphoid organs decrease as a result of OTA, but the weights of the kidney and liver increase due to degenerative alterations.

Huff and hamilton, (1975) stated that Nephrotoxin ochratoxin A causes the kidney to expand, become discoloured, and then accumulate uric acid.

According to Biró *et al*. (2002) told that OTA residues built up in all organs, with the liver and kidneys having the highest concentrations and the muscle having the lowest.

2.9 Tryptophan

Tryptophan is an important amino acid for humans and many other species of animals. It can alter intestinal epithelial cells by producing anti-inflammatory cytokines and can improve the immune system's protection by raising blood levels of immunoglobulin. Tryptophan supplementation in broiler chicken diet models with basal or low crude protein diets enhanced the villus height: crypt depth ratio. According to a recent study, therapy with the tryptophan precursor 5-hydroxytryptophan (5-HTP) boosted the amount of IgA in chickens' duodenum and ileum.

A higher dose of tryptophan is thought to boost growth performance because it affects feed intake, immunity and general health state. Tryptophan was also considered a possible nutrient for in ovo feeding based on this feature.

Tryptophan, a potential nutrient, has been demonstrated by Nayak *et al*. (2018) to have a substantial effect on broiler immune response, productivity improvement, and hatchability by in utero feeding, which enhances the chicks' body weights, immune systems, and overall performance.

Bhanja and Mandal (2005) observed that for chicken, in ovo feeding is essential for enhancing growth performance, FCR, condition of gastrointestinal tract, and immune system. It is a revolutionary strategy for maximising broiler performance while reducing feed prices and the impact they put on the food ecology.

Burley and Vadehra (1989) studied that the amnion of a late-term chicken embryo receives only external nutrients (Uni and Ferket 2003). The broiler breeder hen makes invests in closed nourishment and nutrients for development and growth to promote embryo development and hatching in a medium devoid of carbohydrates and containing a restricted amount of energy to support the development of the bird embryo.

Moran (2007) observed that early embryonic feeding can enhance the energy level and GI tract development of the perinatal bird by adding the amnion with essential nutrients because the avian embryo utilizes amniotic fluid (primarily water as well as albumen protein) orally before pipping out of the air cell around 18 days of incubation in chickens.

Improvements in weight of newly hatched birds and good condition of their immune system lower mortality and morbidity rates, which in turn lower biowaste generation. In order to use in ovo technology, many nutrients must be investigated for accelerating hatchability, hatch weight, and immunity.

MATERIALS AND METHODS

The present investigation was conducted at the Department of Veterinary Clinical Medicine, Odisha University of Agriculture and Technology, Bhubaneswar and in collaboration with RS-DPR Bhubaneswar during month of September to November.

3.1 Experimental eggs and ducklings

A total number of 200 freshly laid eggs of White Pekin ducks were collected from duck shed of RS-DPR, Bhubaneswar where farm is maintained under standard environmental conditions and birds were routinely vaccinated. (Duck plague & Duck cholera vaccine). Sanitization of eggs were done by fumigating them with 35 g of potassium permanganate in 70 ml of 40% formaldehyde solution/m^3 for twenty (20) minutes, than eggs were placed in incubator till inoculation. Eggs were inoculated with different solutions as per experimental design and incubated for 25 days in incubator and rest 3 days in hatcher. After 28 days dead eggs are opened for collection of various body organs for histopathological examination and hatched ducklings were transferred to brooding shed. Health and daily care management were performed routinely in RS-DPR as per standard practices. Ducklings were maintained in deep litter system. Ducklings were provided with sufficient water, ambient temperature, light, humidity etc. Proximal examination of the feed stuff was done before feeding of the ducks. After 28 days blood samples were collected from ducklings for studying haematological and biochemical parameters.

3.2 Experimental design

Table 1: Distribution of eggs in different groups

Group	No. of eggs	In ovo dose of OTA /egg. (0 day of inoculation)	In ovo dose of L-tryptophan/egg (22^{nd} day of inoculation)
G-1 (Control)	50	-	-
G-2 (Tryptophan)	50	-	5 mg/egg
G-3 (OTA)	50	50ng/egg	-
G-4 (OTA+L-Tryptophan)	50	50ng/egg	5 mg/egg

3.3 Preparation of stock solution of Ochratoxin A on 0 day

Pure Ochratoxin was purchased in powder form from Sigma-Aldrich Co.(cat. no O1877-1MG) and than we have prepared the stock solution of Ochtatoxin A, after proper dilution of OTA powder using methanol and 0.1% M NaHCO3 in concentrations of 10 ng/µl and further diluted to get concentration of 50 ng/10 µl as we have to inoculate desired level (50 ng) of toxin in 10 µl solution/egg. Analysis of OTA concentration in prepared solution was done by using Ochra test in sensitive VICAM flurometer.

3.4 Inoculation of eggs with OTA solution on 0 day of incubation

For inoculation aseptic environment is prepared, 100 eggs are taken out from incubator for inoculation, eggs are again wiped with cotton and air sac area was marked. After drilling the eggs at broad end (towards air sac), 10 µl of OTA stock solution was injected on air cell membrane using micropipettes with sterile tips (Vesla *et al.*, 1983). After inoculation the drilled holes were quickly sealed with wax. The eggs were again placed in an incubator cum hatcher maintained at 37.8°C temperature and 65% relative humidity and turned through 270°C every 2 hours.

Eggs are monitored everyday for temperature, humidity, proper turning. The dead eggs are removed by candling and mortality is recorded.

3.5 Preparation of stock solution of tryptophan on 22nd day

Pure grade Tryptophan (Tokyo Chemical Industries Japan, Lot. 3FOGF-PD) is diluted in sterile N.S. solution in such a way that we can get final concentration of 5 mg Tryptophan/10 µl of solution, Tryptophan is dissolved properly using water bath and vortex mixer.

3.6 Inoculation of eggs with tryptophan solution on 22nd day of incubation

After preparation of solution eggs of group 3 and group 4 are taken out are taken out and are wiped using cotton, air sac area was marked. After drilling the eggs at broad end (towards air sac), 10 µl of Tryptophan stock solution was injected through amniotic route using sterile needle and syringe. After inoculation the holes were sealed quickly with melted wax. The eggs were again placed in an incubator maintained at 37.8°C and 65% relative humidity and turned through 270°C every 2 hours. After completion of 25 days eggs are transferred to hatcher maintained at temperature of 37.2°C and 75% relative humidity.

After hatching live ducklings were marked using wing band and weighed using calibrated digital weighing balance and transferred to brooding shed which is maintained under standard brooding condition.

3.7 Calculation of hatchability percentage

$$\text{Hatchability} = \frac{\text{Number of eggs hatched}}{\text{Total number of hatching eggs}} \times 100$$

3.8 Collection of tissue samples for studying histopathological changes in embryos

Unhatched eggs were collected and are opened for collection of various organ (liver, kidney, bursa, thymus) for histopathological examination in 10% buffered neutral formalin solution.

3.9 Measurement of body weight

Body weights were recorded at weekly intervals upto 4 weeks of age using calibrated digital weighing balance.

3.10 Collection of samples

Collection of blood samples after 28 days for the study of haematological and biochemical parameters of various groups. Blood samples were collected from medial tarsal vein of ducklings through different commercially available vials like clot activator and EDTA vials for blood biochemical parameters and haematological study respectively.

All the experimental birds were kept under proper supervision for showing any clinical signs and symptoms of toxicity like dullness, lameness and others including mortality if any occurred. All handling of toxins was done under biosafety procedures. The experimental protocol was conducted as per Institutional Animal Ethics Committee (IAEC) guidelines.

3.11 Study of haematological changes in ducklings

The peripheral venous blood was collected aseptically in sterile test tubes from medial tarsal vein by using 24 gauge needles. For haematological study, blood was collected in sterile vial using EDTA as anti-coagulant @1.0mg/5ml of blood as recommended by Jain (1986) and it was brought to Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. TEC and TLC was determined by using flow cytometry/microdilution method. The PCV value was estimated by Microhematocrit capillary tube method. Estimation of Hb was done by Sahli's acid haematin method and differential count (Heterophils, Eosinophils, Basophil, Lymphocytes and Monocyte) by modified Giemsa stain.

3.12 Study of serum biochemical changes in ducklings

The collected serum samples were brought to Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar for different biochemical assays in spectrophotometer (EPPENDORF) using commercial kits ($CORAL^R$). These parameters get estimated Total protein, albumen, Globulin, Triglycerides, cholesterol, urea, creatinine, AST (SGOT), ALT (SGPT).

3.13 Histopathological study

Representative tissue samples collected from different organs during necropsy. The size of the specimen was 4mmx4mmx4mm for a solid tissue. Fixation of tissue samples was done in a container carrying fixing solution such as neutral formalin at least 10 times the volume of tissue for more than one month.

Tissue processing for histopathology

Removed the tissues from fixing solution and wash under running tap water overnight to remove the formalin from tissue. Blot the tissue after removal from washing step and treat with dehydrating agents *i.e.* ascending grades of alcohol like 70%, 80%, 90% and 100% for fixed time. Clearing was done with use of Xylene for fixed time as per routine haematoxyline and eosin staining procedure. Once clearing process was completed, then infiltration / impregnation process started with adding one medium *i.e.* Paraffin into the tissue cut sections which was done by immersing the cut tissue sections in molten paraffin with certain temperature and time protocol. After that tissue embedding through Paraffin wax was conducted by using two brass modules / plates of L-size. Arranging these two L-shaped brass plates / moulds so as to form a well into which molten paraffin was poured and kept inside an oven with certain time and temperature protocol. With the help of warmed forceps, lifting of the tissue from paraffin cup-and simultaneously pressing at the bottom of the well with a gentle pressure in correctly oriented plane was made with proper labelling / tag for easy identification of the tissue sections. As soon as a thin solid wax film is formed on the surface, the whole mould/ paper boat set may be submerged beneath cold water for preventing the formation of large paraffin crystals inside the cut sections of tissue. After then, brass moulds were removed on solidification of the paraffin.

Trimming was done for removal of excess paraffin from the tissue blocks. The tissue surface towards the base of mould is the surface from which sections are to be cut; this surface was trimmed lightly to expose the tissue. The opposite surface, to be attached to the block holder of the microtome, was trimmed parallel to the first. All the four sides of the block were trimmed keeping their opposite edges to each other such that a minimum of wax remained between the tissue and edge. Sectioning of the paraffin embedded tissue sections were done manually by using a hand operated microtome, present in the Department of veterinary Pathology of the college. Tissue slices of 5-7 micro meters were obtained in the form of ribbon. After selecting the best section from the ribbon, it was allowed to be released on hot water bath maintained at 45ºC for proper flattening for 1-2 minutes. Later on, it was lifted on an egg-albumenized micro-slide which was kept on its edge in slanting position for draining of water and air drying. Drying the slides in an oven maintained at 40ºC over-night and store at dry place in slide rack for staining purpose through routine haematoxylin and eosin staining (H&E).

3.14 Statistical analysis

The effect of Ochratoxin on growth, haematological and biochemical parameters of ducklings in comparison to ducklings of control, Tryptophan, Ochratoxin A+ Tryptophan supplemented groups was analysed by Multivariate Analysis of Variance (ANOVA) using statistical Package For Social Science (SPSS), International Business Machines (IBM) Armonk New York and the experimental and control groups difference were considered statistically significant at $P \leq 0.01$ and $P \leq 0.05$. The graphs were prepared using software, Graph Pad Prism v4.03 (San Diego, CA, USA).

Fig. 1: Collection of eggs **Fig. 2: Candling of eggs**

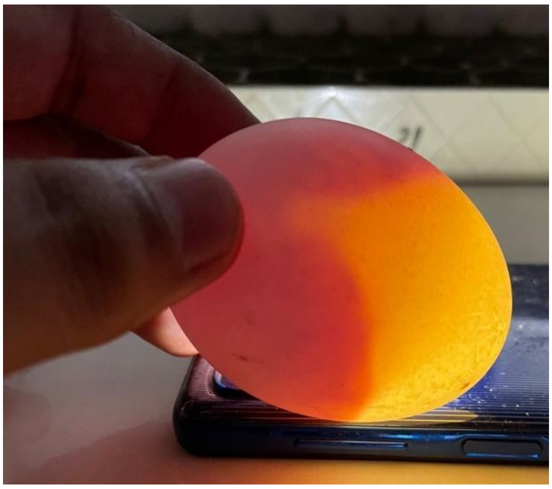


Fig. 3: Live egg **Fig. 4: Dead egg**

Fig. 5: Preparation of stock solution of OTA using methanol and $NaHCO_3$

Fig. 6: Estimation of toxin concentration in stock OTA solution using ochra test column

Fig. 7: Inoculation of OTA solution

Fig. 8: Inoculation of Tryptophan solution

Fig. 9: Driller for making hole inn egg

Fig. 10: Sealing of hole using wax

Fig. 11: Regular monitoring of eggs and removal of dead eggs

Fig. 12: Measurement of body weight of hatched ducklings

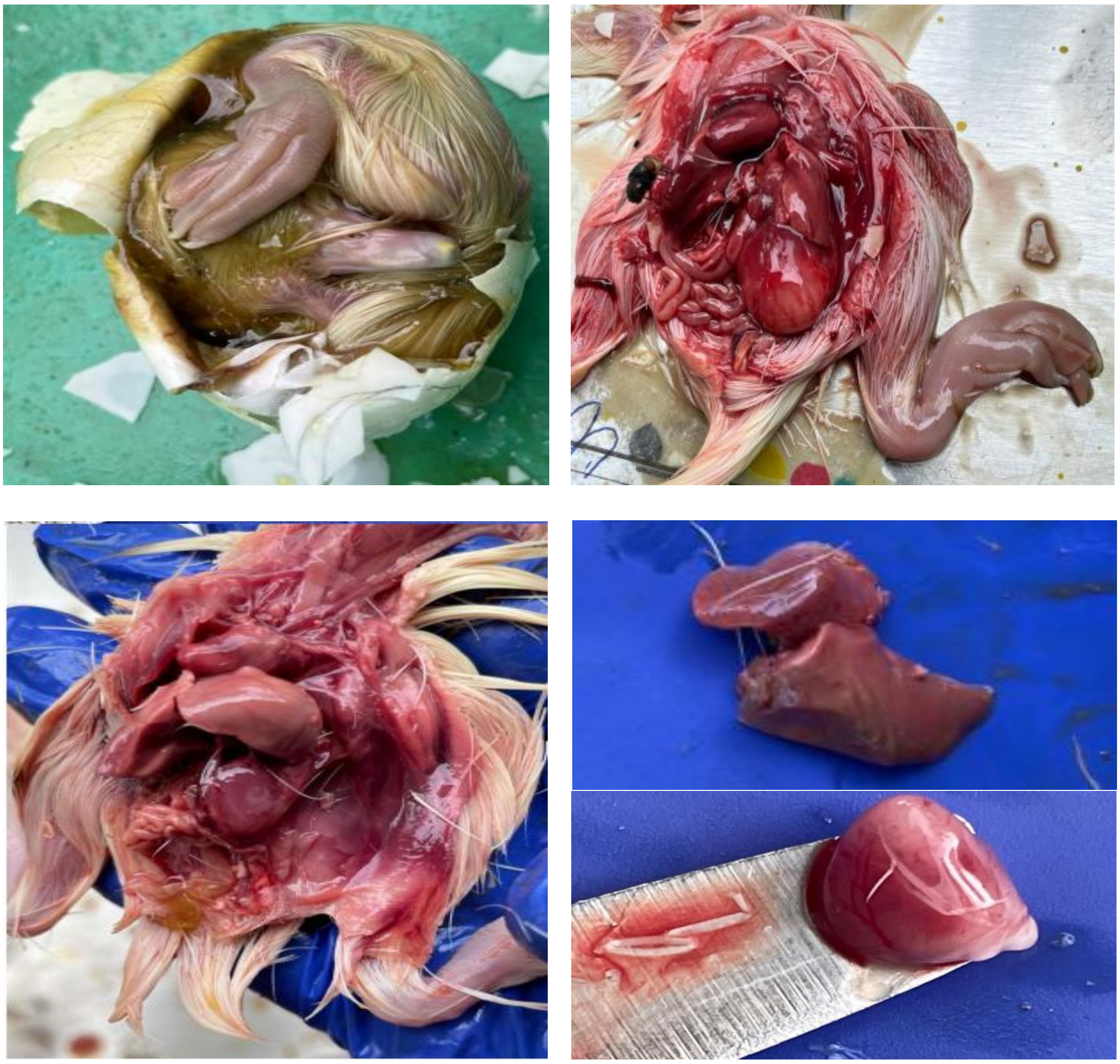


Fig. 13: Opening of dead eggs for organ collection

Fig. 14: Embryos of different groups

Fig. 15: Collection of blood for haematology and biochemical parameters

Fig. 16: Estimation of serum biochemical parameters using spectrophotometer

Fig. 17: Tissue section cutting using microtome

Fig. 18: Paraffin embedding and block making

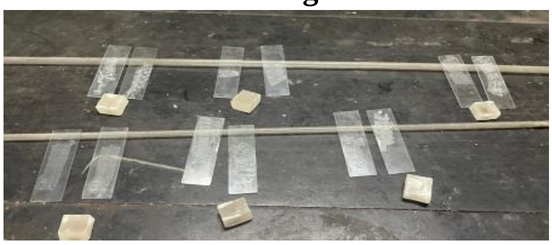


Fig. 19: H&E stained slides

RESULTS

4.1 Effect of Tryptophan on hatchability in ducklings exposed to OTA

After completion of incubation period, maximum hatchability was seen in control group (G-1) followed by Trp (G-2) group and than OTA+Trp group (G-4) while least hatchability was seen in OTA(G-3) treated groups, the percentage of hatchability in different groups G-1, G-2, G-3 and G-4 are (70,66,20 and 40) respectively.

4.2 Effect of Tryptophan on weekly body weight in ducklings exposed to OTA

On 0th day of hatching it was observed that there was a significant difference in body weight of ducklings in OTA treated group (G-3) in comparison to control (G-1) and Trp group (G-2). Group 2 containing Trp has shown highest body weight whereas 3rd group containing OTA has lowest body weight of ducklings. On completion of 4th week, there was significant difference ($P<0.01$) in body weight in OTA treated group (G-3) in comparison to control group (G-1) and Trp group (G-2). However, body weight in OTA+Trp group (G-4) is higher than OTA group (G-3) which shows in-ovo Tryptophan improves growth performance and has ameliorative effect over OTA infected birds.

Table 2: Effect of L-Tryptophan on weekly body weight in ducklings exposed to OTA

Body weight	Group I (Control)	Group II (Tryptophan)	Group III (OTA)	Group IV (OTA+ Tryptophan)	Significance
0 Day	43.53±1.52^{b}	46.37±1.52^{b}	39.86±1.10^{a}	43.00± 0.58^{ab}	P<0.01
1st Week	82.67±1.67^{c}	87.27±1.29^{c}	67.00±1.18^{a}	77.00±1.46^{b}	P<0.01
2nd Week	139.83±3.62^{bc}	152.08±5.15^{c}	105.48±1.61^{a}	132.78±9.23^{b}	P<0.01
3rd Week	666.83±24.21^{b}	731.50±31.52^{b}	543.50±29.09^{a}	662.33±39.31^{b}	P<0.01
4th Week	1131.67±36.75^{bc}	1194.83±29.97^{c}	913.17±22.59^{a}	1059.33±35.74^{b}	P<0.01

Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

4.3 Effect on haematological parameters

4.3.1 Effect of Tryptophan on Hb, PCV, TEC in ducklings which are exposed to OTA

Ducklings hatched out from OTA inoculated eggs showed least values of Hb, PCV, TEC, in comparison to other groups, whereas the group 4 which is treated with both OTA on 0 day and Trp on 22nd day showed ameliorative effect due to tryptophan. Maximum values of Hb, PCV, TEC, are shown by group 2 which is treated by Trp only on 22nd day and which was significantly higher than control group.

Table 3: Effect of L-Tryptophan on hematological parameters in ducklings exposed to OTA

Parameters	Group I (Control)	Group II (Tryptophan)	Group III (OTA)	Group IV (OTA + Tryptophan)	Significance
Haemoglobin (g%)	16.68 $\pm 0.28^{b}$	18.45 $\pm 0.08^{c}$	13.95 $\pm 0.20^{a}$	16.50 $\pm 0.14^{b}$	P<0.01
PCV (%)	43.50 $\pm 1.86^{a}$	53.83 $\pm 0.60^{b}$	42.00 $\pm 1.15^{a}$	46.00 $\pm 1.93^{a}$	P<0.01
TEC (RBC$\times 10^{12}$)	3.65 $\pm 0.19^{ab}$	4.22 $\pm 0.05^{b}$	3.24 $\pm 0.32^{a}$	3.50 $\pm 0.13^{a}$	P<0.05
TLC (Cells/mm^3)	50166.67 $\pm 945.75^{b}$	54000.00 $\pm 683.13^{c}$	44666.67 $\pm 760.12^{a}$	48000.00 $\pm 966.09^{b}$	P<0.01
Heterophil$\times 10^9$/l	28.17 ±0.48	30.50 ±0.62	26.50 ±0.43	30.33 ±2.49	NS
Basophil$\times 10^9$/l	1.00 ±0.36	1.50 ±0.43	1.67 ±0.42	1.33 ±0.33	NS
Eosinophil$\times 10^9$/l	5.17 ±0.48	5.50 ±0.34	4.67 ±0.49	4.50 ±0.85	NS
Lymphocyte$\times 10^9$/l	58.50 ±1.12	55.50 ±1.52	54.83 ±1.64	55.17 ±0.95	NS
Monocyte$\times 10^9$/l	6.50 $\pm 1.06^{a}$	10.00 $\pm 0.89^{b}$	11.00 $\pm 0.45^{b}$	9.00 $\pm 0.36^{ab}$	P<0.01

Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

4.3.2 Effect of Tryptophan on Total Leukocyte Count (TLC), Differential leuckocyte count (DLC) in ducklings which are exposed to OTA

No significant difference was found between the values of heterophils, basophils, eosinophils and lymphocytes in all 4 groups, although there was a significant difference ($P<0.01$) observed in the value TLC, which was higher in OTA treated group (G-3) as compared to control group (G-1) and Trp group (G-2). The TLC value of OTA+T group (G-4) is significantly higher than OTA treated group (G-3) which shows the ameliorative effect of L-Tryptophan. The serum monocyte levels in OTA group (G-3) is significantly higher than control group (G-1) whereas no significant difference is seen in values of monocytes in OTA treated group (G-3) and Trp group (G-2). There was no significant difference between the Monocyte values of OTA (G-3) group and OTA+T group (G-4) which tells L-Tryptophan has no effect on monocytes of OTA treated birds.

4.4 Effect on biochemical parameters

4.4.1 Effect of Tryptophan on glucose, cholesterol and triglyceride in ducklings which are exposed to OTA

No significant difference is found in serum levels of glucose, cholesterol and triglyceride in all four groups it means OTA and Trp do not affect these biochemical parameters.

4.4.2 Effect of Tryptophan on kidney (BUN, Creatinine, Uric acid) parameters in ducklings which are exposed to OTA

After biochemical analysis it was found that serum BUN, creatinine and uric acid level shows significant difference in group 1, 2 and 3.OTA inoculated group (G-3) ducklings showed maximum serum levels of BUN, creatinine and uric acid in comparison to other groups which shows the nephrotoxic effect of OTA in ducklings. Whereas value of BUN, creatinine and uric acid in OTA+T group (G-4) group is lower than OTA group (G-3) which shows ameliorative effect of Tryptophan over Ochratoxin infected ducklings.

4.4.3 Effect of Tryptophan on (Total protein, Albumin and Globulin) in ducklings which are exposed to OTA

On biochemical study significant difference (P<0.05) was seen in levels of Total protein and globulin. In comparison to control group (G-1) group 3 chicks which are inoculated with OTA showed least value of Total protein and globulin whereas maximum value was evident in Trp group (G-2). No significant difference was seen in values of albumin in all 4 groups.

4.4.4 Effect of Tryptophan on Liver (SGOT, SGPT) parameters in ducklings which are exposed to OTA

After biochemical analysis a significant difference in SGOT and SGPT values are evident in group 1, 2 and 3. OTA inoculated group (G-3) ducklings showed maximum serum levels of SGOT and SGPT in comparison to other groups which shows the hepatotoxic effect of OTA in ducklings. Whereas value of SGOT and SGPT in OTA+T group (G-4) group is lower than OTA group (G-3) which shows ameliorative effect of Tryptophan over Ochratoxin infected ducklings.

Table 4: Effect of L-Tryptophan on biochemical parameters in ducklings exposed to OTA

Parameters	Group I (Control)	Group II (Tryptophan)	Group III (OTA)	Group IV (OTA+ Tryptophan)	Significance
Glucose (mg/dl)	61.91±4.97	68.14±1.54	65.44±1.26	62.37±2.66	NS
Triglyceride (mg/dl)	65.34±1.52	66.05±1.25	61.55±2.00	64.95±2.15	NS
Cholesterol (mg/dl)	109.66±2.18	116.25±5.49	124.97±2.61	119.74±19.70	NS
BUN (mg/dl)	16.64±0.36^{a}	17.66±0.30^{a}	19.42±0.64^{b}	17.19±0.31^{a}	P<0.01
Creatinine (mg/dl)	0.18±0.02^{a}	0.18±0.02^{a}	0.57±0.05^{c}	0.39±0.05^{b}	P<0.01
Uric Acid	4.63±0.25^{a}	4.73±0.29^{a}	12.02±1.01^{c}	10.02±1.91^{b}	P<0.01
SGOT (U/L)	73.92±9.18^{a}	70.93±4.45^{a}	127.09±8.00^{b}	85.52±6.85^{a}	P<0.01
SGPT (U/L)	95.36±6.77^{b}	65.21±5.06^{a}	268.31±13.39^{c}	70.76±4.29^{a}	P<0.01
Total Protein (g/dl)	2.74±0.06^{bc}	3.10±0.20^{c}	2.34±0.09^{a}	2.57±0.21^{b}	P<0.05
Albumin (g/dl)	1.32±0.05	1.38±0.07	1.26±0.05	1.28±0.03	NS
Globulin (g/dl)	1.37±0.05^{bc}	1.78±0.17^{c}	1.07±0.12^{a}	1.30±0.18^{b}	P<0.05

Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

4.4 Pathological findings

4.4.1 Gross pathology

The White Pekin duck embryos (n=18, each group @ 6) pertaining to three experimental groups (n=3) were collected from un-hatched eggs after completion of incubation period (29^{th} day) for detailed necropsy examinations. The representative tissue samples of size 4mmx4mmx4mm were collected and fixed in 10% neutral formalin for further processing. The detail pathomorphological changes in different organs such as liver, kidney and thymus were recorded.

Group-II (Only tryptophan)

The white pekin duck eggs were inoculated with only tryptophan @5mg/egg on 22^{nd} day through amniotic route by single window opening technique experimentally to observe any pathomorphological changes in different organs. Detail necropsy lesions were recorded which showing well developed thymus without any structural abnormalities (Fig. 38), pale liver (Fig. 39) and kidney with near to normal size and shape (Fig. 40) in majority of dead embryos. Very few showing slight enlargement of hepatic parenchyma with sharp edges (Fig. 41). Detail post-mortem examinations revealed absence of hydrocephalus

Group-III (Only OTA)

Ochratoxin - A (OTA) injected *in-ovo* on Zero (0^{th}) day @ 50 ng/egg through air cell membrane route by single window opening technique. The embryos were showing conclusive abnormal growth (Fig. 42) during necropsy with some embryos showing hydrocephalus (Fig. 43) conditions. Most of the kidneys were deeply congested and highly reticulated (Fig. 44). There was diffuse congestion with severe enlargement of kidney noticed during post-mortem examination. Similarly, there was severe hepatomegaly with haemorrhages and congestion (Fig. 45). Majority of the duck embryos were showing ill-developed thymus (Fig. 46) or atrophied thymus. In some of the embryos, the liver was enlarged to 4 to 5 times as compared to other groups of similar age groups with sharp round edges. Such enlarged liver (Fig. 47) was showing petechial haemorrhages along with congestion and easily friable with presence of some blood clots. Two of the dead embryos were showing ascites with blood-tinged fluid accumulations in the peritoneal cavity.

Group-IV (OTA with tryptophan)

Tryptophan considered as an important essential amino acid inoculated to the OTA injected eggs @ 5 mg/egg on 22nd day through amniotic route with an intention to find any ameliorative effect on the embryos. Necropsy examination of the duck embryos revealed a slight ameliorative effect with characteristically well-developed thymus with minimal hydrocephalus (Fig. 48) condition as compared to Group-III (Only OTA). There was mild enlargement of kidney with minimal congestion (Fig. 49) suggestive of reduced nephropathy as compared to previous group with OTA inoculation only. Similarly, mild hepatomegaly (Fig. 50) was evident in these groups during detail post-mortem examination.

4.4.2 Histopathology

The organs collected during necropsy were processed for routine haematoxylin and eosin staining (H&E) in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar through Paraffin embedding technique. Representative tissue samples from liver, kidney, thymus, bursa of fabricius were fixed in 10% formalin for two months and then processed for routine H&E staining.

Group-II (Only tryptophan)

Histopathological lesions as observed in the dead embryos inoculated with tryptophan@5mg/egg on 22nd day showed very minimal histological alterations as observed through microscopy related to kidney. There were intact glomeruli with normal tubular epithelial linings (Fig. 51) without any architectural disorientations and infiltrations of any inflammatory cells. There was no such narrowing of the tubular lumen with absence of any proteinaceous exudates. However, the histological lesions related to hepatic parenchyma showed vacuolar degenerations with heavy infiltrations of inflammatory cells (Fig. 52) and congestion.

Group-III (Only OTA)

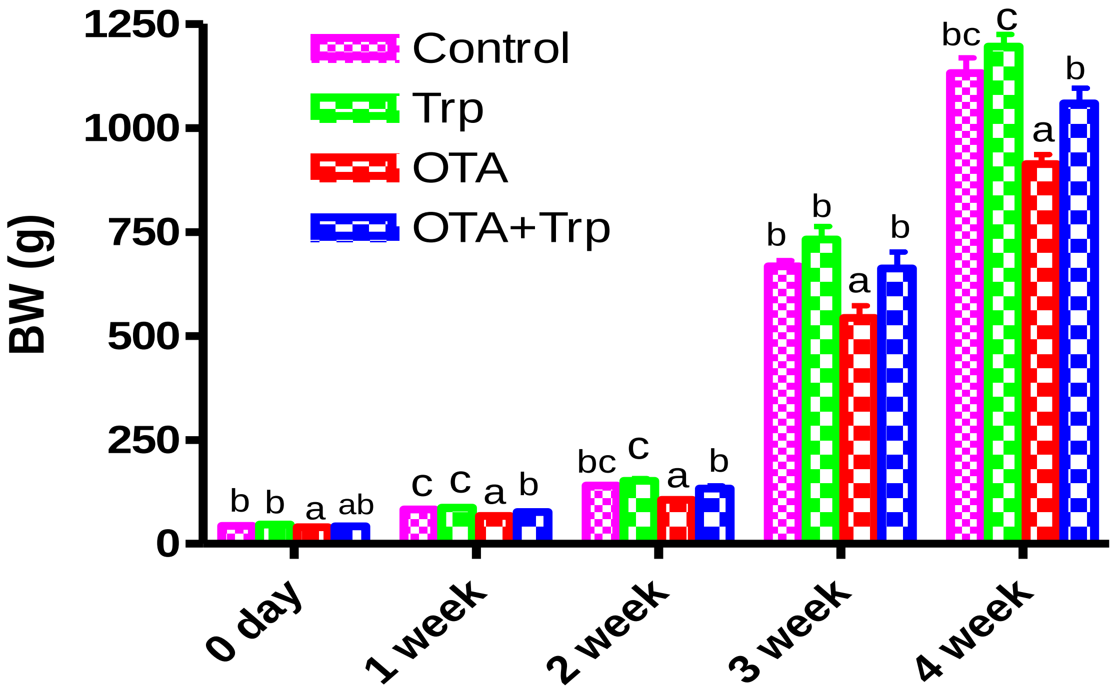
In-ovo inoculation of Ochratoxin -A (OTA) on Zero (0th) day @ 50ng/egg in the White Pekin duck eggs showed major pathological changes as evidenced in the dead embryos collected during necropsy on 29th day.

Histologic alterations in liver were predominantly characterised by disorganization of hepatocytes (Fig. 53) with degenerations and necrosis of hepatocytes besides diffused infiltrations of mononuclear cells and presence of edema fluids. Majority of hepatic parenchyma were showing dissolution of hepatic architecture with presence of few pyknotic nuclei (Fig. 54) and minimal inflammatory cells. There was presence of small to large clear spaces / vacuoles resembling vacuolar degenerations (Fig. 55) with mild sinusoidal congestion (Fig. 56) with necrosis of hepatic parenchyma. In majority of cases, diffused infiltrations of inflammatory cells along with congestion were observed as prominent histologic lesions. Microscopic alterations related to kidney were sub-capsular infiltrations of mononuclear cells (Fig. 57) necrosis of renal tubular epithelial cells, disorganization and / or dissolution of renal tubular epithelial lining cells and congestion (Fig. 58). Histopathologic lesions in some cases were showing tubular necrosis, narrowing of tubular lumen, mononuclear cells infiltrations in the inter tubular areas with desquamation of tubular cells evident inside the tubular lumens (Fig. 59). Lymphoid organs like Bursa of Fabricus and thymus were collected and processed for histopathology which was showing depletion of bursal (Fig. 60) and thymic follicles.

Group-IV (OTA with tryptophan):

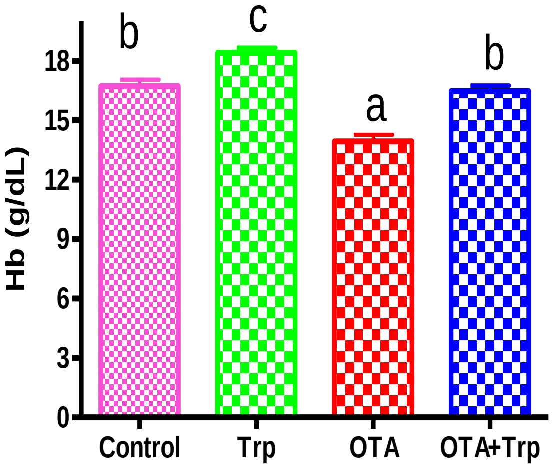
Organs collected from the experimental group inoculated with Ochratoxin-A (OTA) along with tryptophan showed mild to moderate histological alterations as evidenced through microscopy. Microscopic changes related to liver as observed were hepatocellular degenerations (Fig. 61), mild infiltrations of inflammatory cells and moderate congestion. In Kidney, there was mild cellular degeneration, mild to moderate tubular degeneration (Fig. 62) with minimal infiltrations of inflammatory cells (Fig. 63). Few cases showing narrowing of the tubular lumen with congestion (Fig. 64). No such abnormalities noticed during microscopy of thymus parenchyma in these group inoculated with Ochratoxin-A and L-tryptophan.

Fig. 20: Effect of Tryptophan on body weight in ducklings exposed to OTA



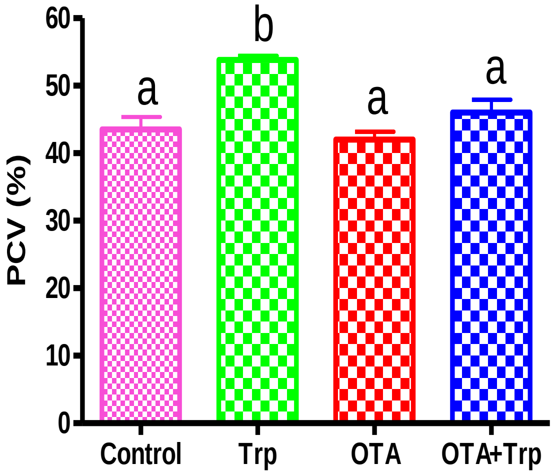
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 21: Effect of Tryptophan on haemoglobin in ducklings exposed to OTA



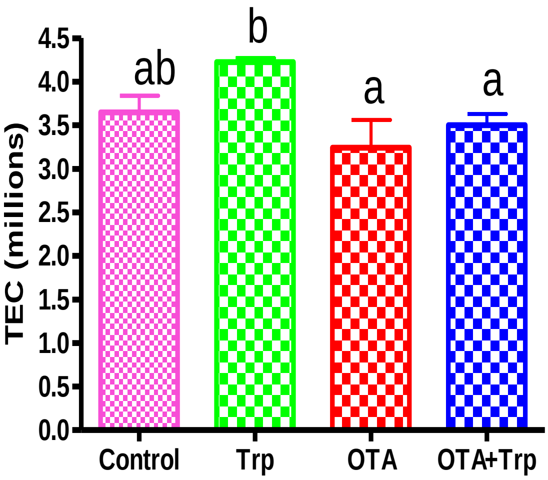
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 22: Effect of Tryptophan on PCV in ducklings exposed to OTA



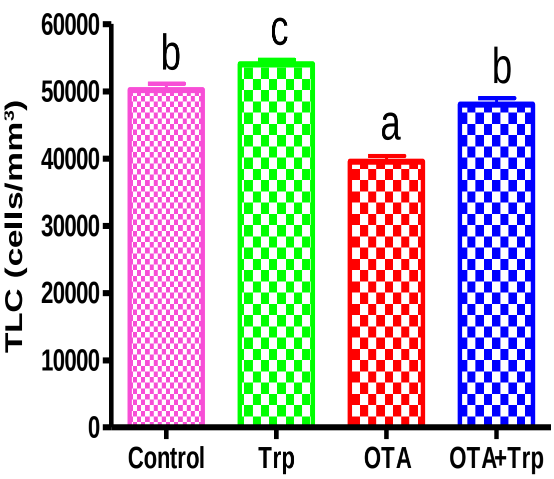
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 23: Effect of Tryptophan on TEC in ducklings exposed to OTA



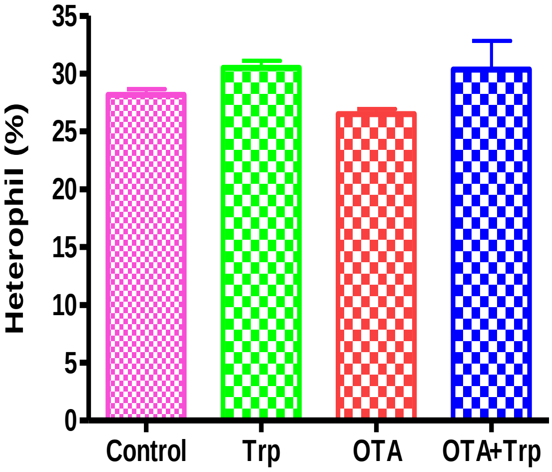
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 24: Effect of Tryptophan on TLC in ducklings exposed to OTA



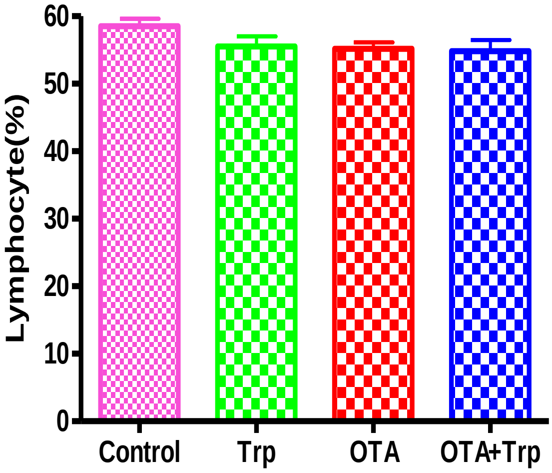
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 25: Effect of Tryptophan on heterophil in ducklings exposed to OTA



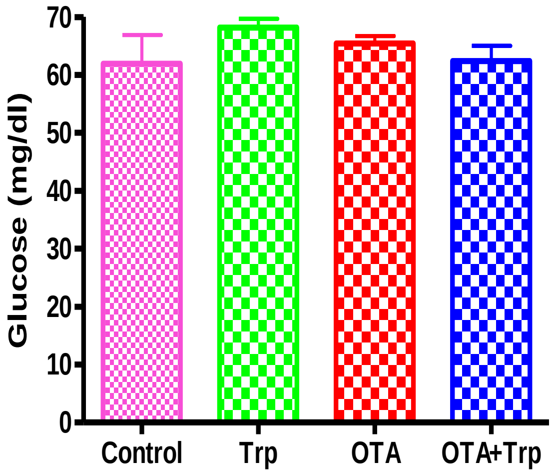
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 26: Effect of Tryptophan on lymphocyte in ducklings exposed to OTA



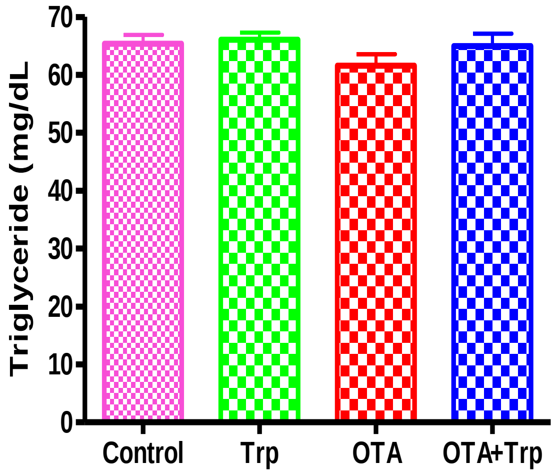
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 27: Effect of Tryptophan on glucose in ducklings exposed to OTA



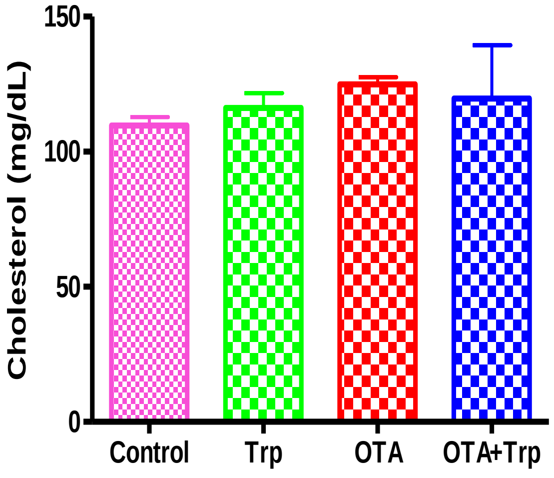
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 28: Effect of Tryptophan on Triglyceride in ducklings exposed to OTA



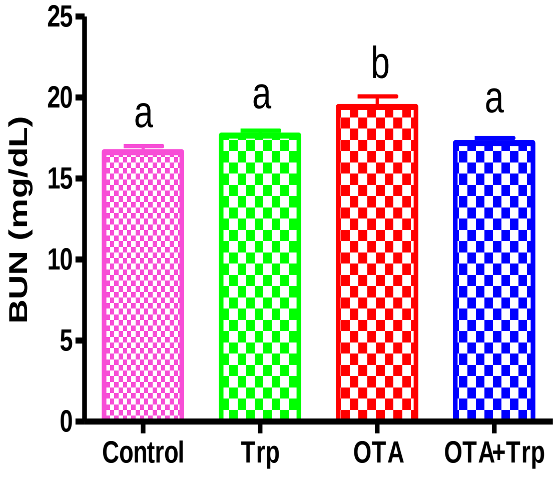
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 29: Effect of Tryptophan on cholesterol in ducklings exposed to OTA



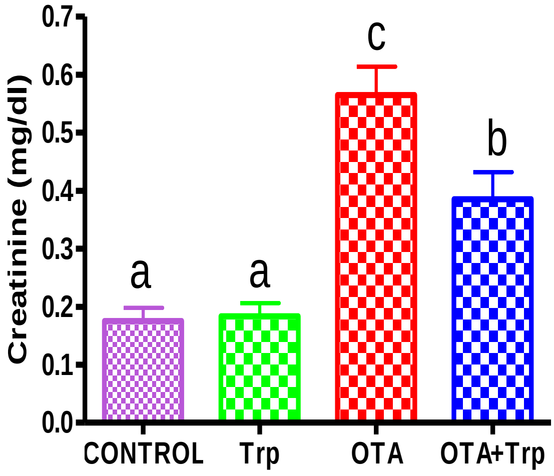
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 30: Effect of Tryptophan on BUN in ducklings exposed to OTA



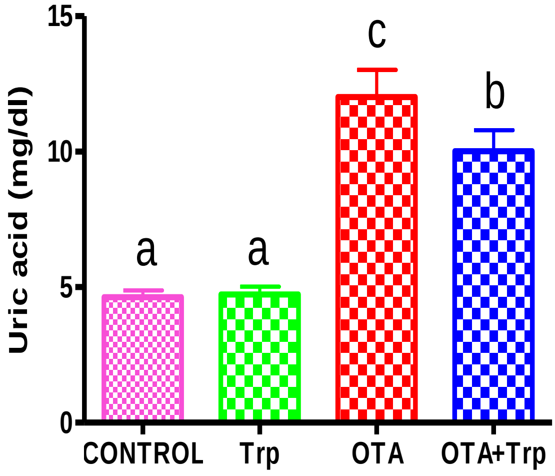
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 31: Effect of Tryptophan on creatinine in ducklings exposed to OTA



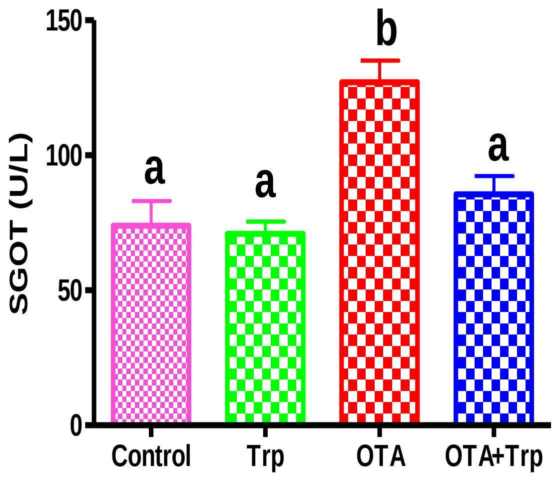
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 32: Effect of Tryptophan on uric acid in ducklings exposed to OTA



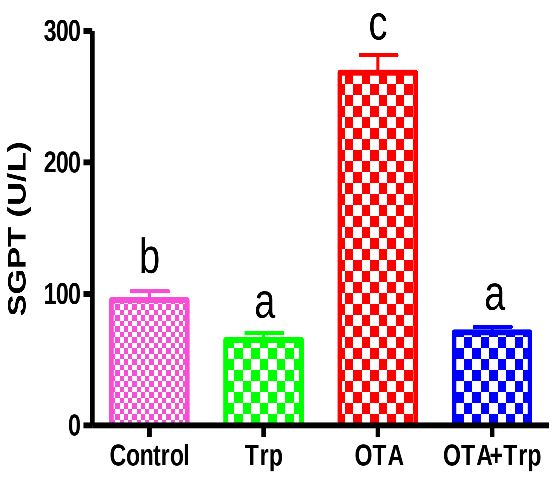
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 33: Effect of Tryptophan on SGOT in ducklings exposed to OTA



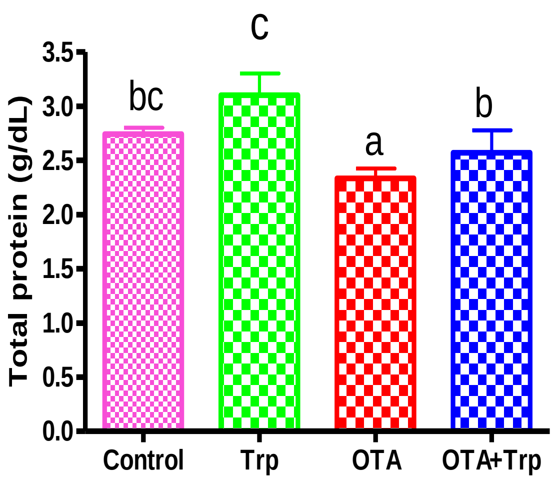
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 34: Effect of Tryptophan on SGPT in ducklings exposed to OTA



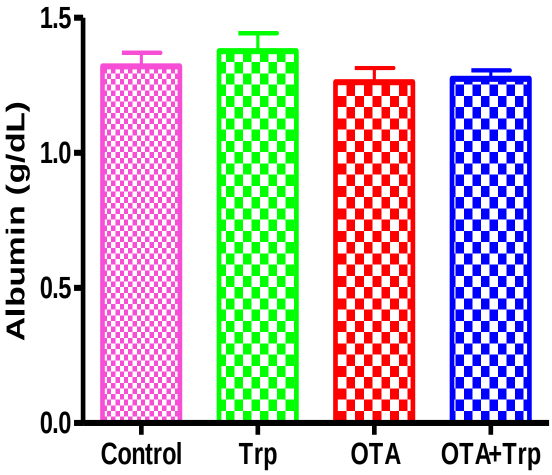
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 35: Effect of Tryptophan on total protein in ducklings exposed to OTA



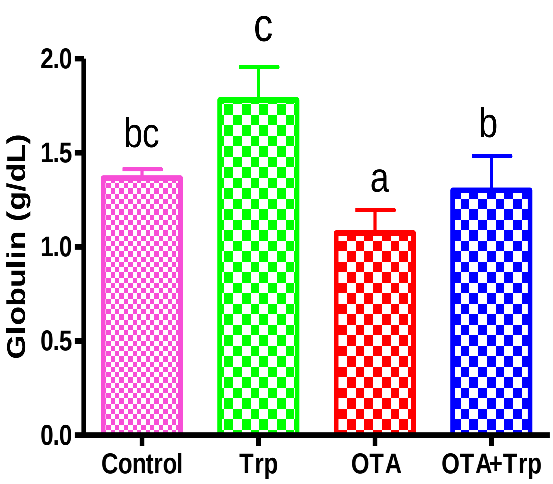
Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 36: Effect of Tryptophan on albumin in ducklings exposed to OTA



Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)

Fig. 37: Effect of Tryptophan on globulin in ducklings exposed to OTA



Data are expressed as mean ± SE; n=6; Means bearing dissimilar superscripts across the column differ significantly (P<0.01)


Fig. 38: Well developed thymus without any structural abnormalities

Fig. 39: Pale liver


Fig. 40: Kidney in normal shape and size

Fig. 41: Slight enlargement of hepatic parenchyma with sharp edges


Fig. 42: Embryo showing abnormal growth

Fig. 43: Embryo head showing hydrocephalus

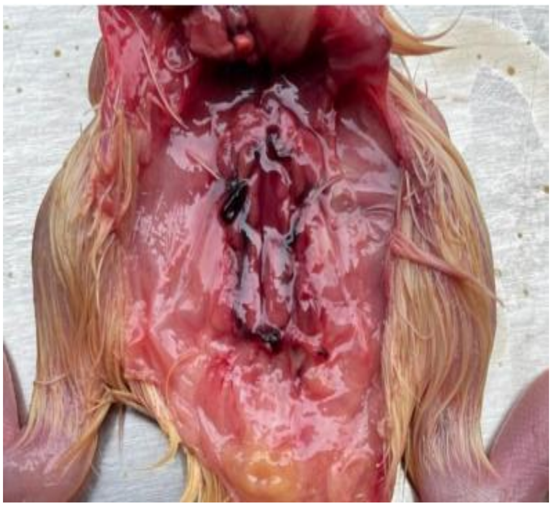


Fig. 44: Deeply congested and highly reticulated kidney

Fig. 45: Hepatomegaly with haemorrhages

Fig. 46: Ill developed thymus

Fig. 47: Enlarged liver with petechial haemorrhages and congestion

Fig. 48: Minimal hydrocephalus in OTA+Trp group

Fig. 49: Mild enlargement of kidney with minimal congestion

Fig. 50: Mild hepatomegaly

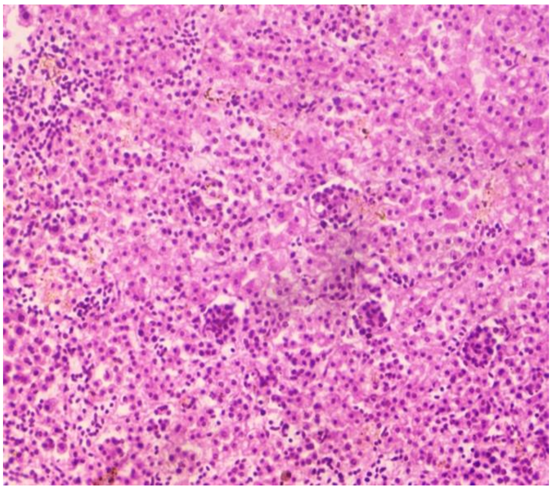


Fig. 51: Intact glomeruli with normal tubular epithelial lining

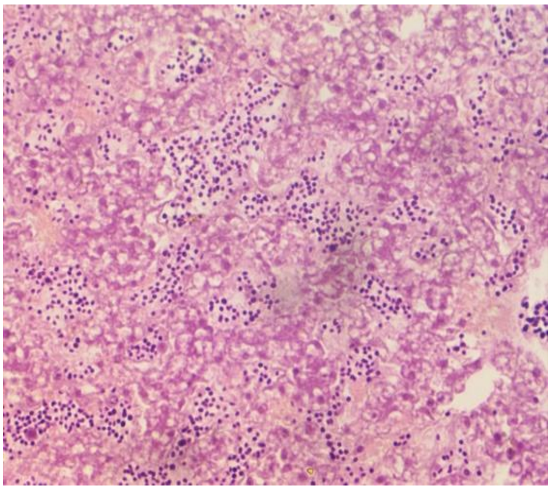


Fig. 52: Hepatic parenchyma showing vacuolar degeneration, congestion and heavy infiltration of inflammatory cells

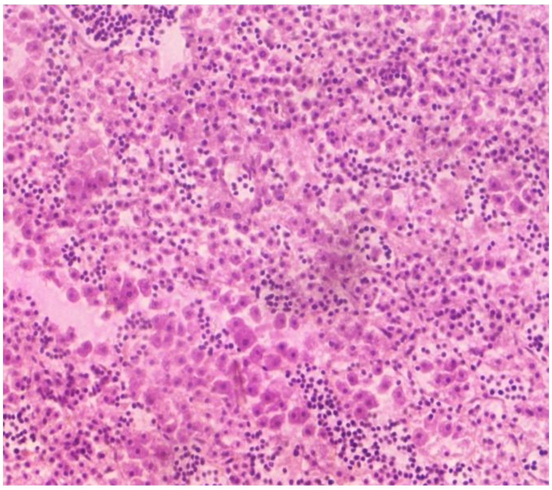


Fig. 53: Disorganization, degenerations and necrosis of hepatocytes. Diffused infiltrations of mononuclear cells and presence of edema fluids

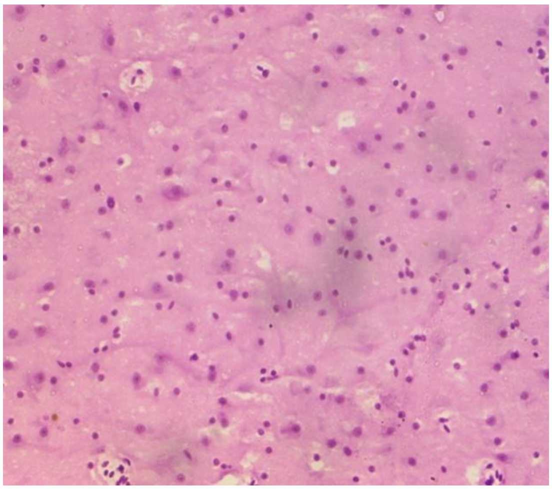


Fig. 54: Dissolution of hepatic architecture with presence of few pyknotic nuclei and minimal inflammatory cells

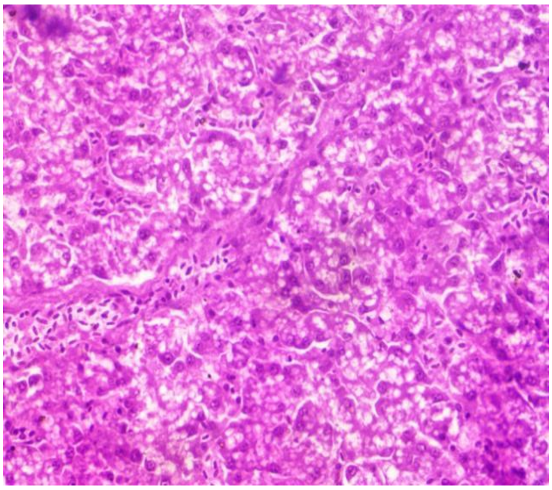


Fig. 55: Presence of small to large clear spaces and vacuoles resembling vacuolar degenerations with mild sinusoidal congestion

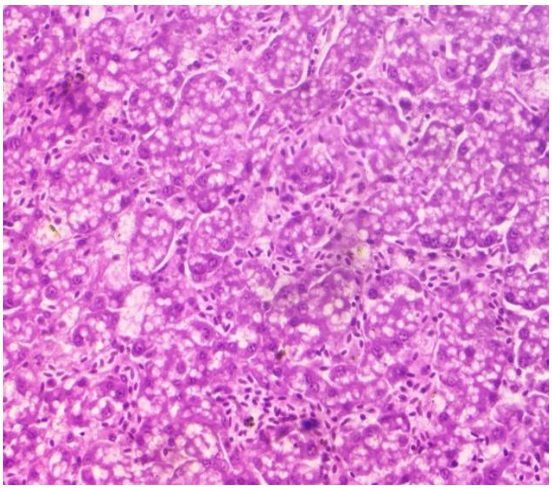


Fig. 56: Necrosis of hepatic parenchyma

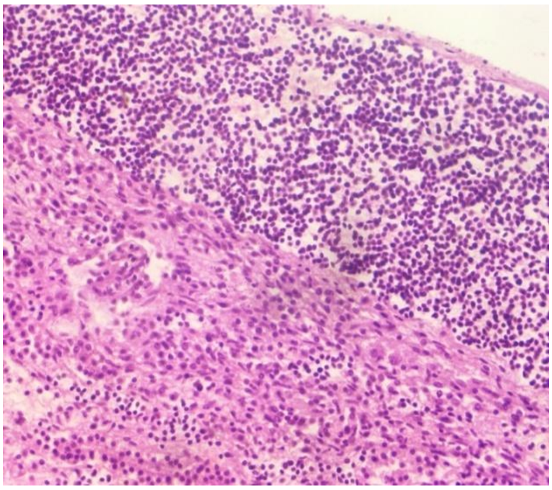


Fig. 57: Sub-capsular infiltrations of mononuclear cells in kidney

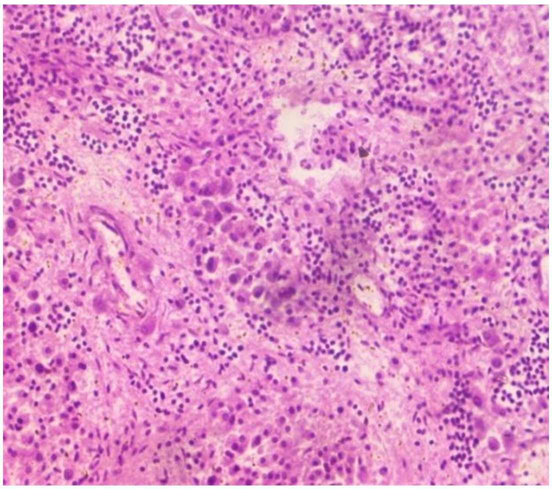


Fig. 58: Necrosis of renal tubular epithelial cells, disorganization and dissolution of renal tubular epithelial lining cells and congestion

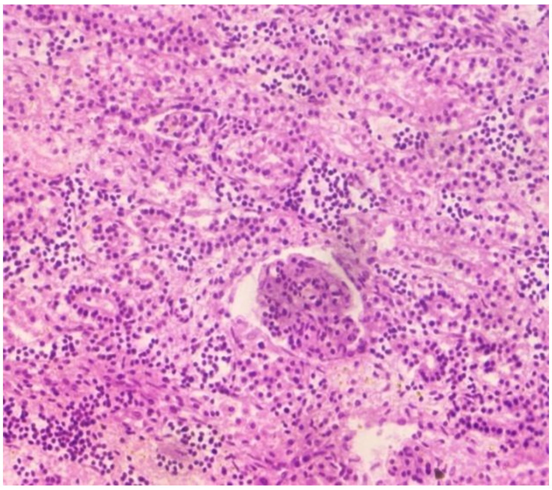


Fig. 59: Tubular necrosis, narrowing of tubular lumen, mononuclear cells infiltrations in the inter tubular areas with desquamation of tubular cells evident inside the tubular lumens

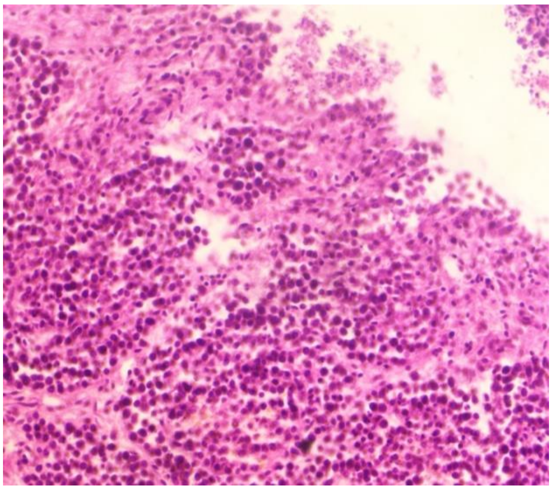


Fig. 60: Depletion of bursal and thymic follicles

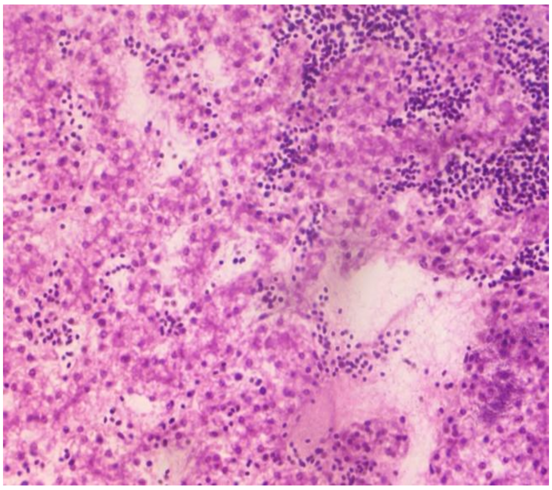


Fig. 61: Hepatocellular degenerations mild infiltrations of inflammatory cells and moderate congestion

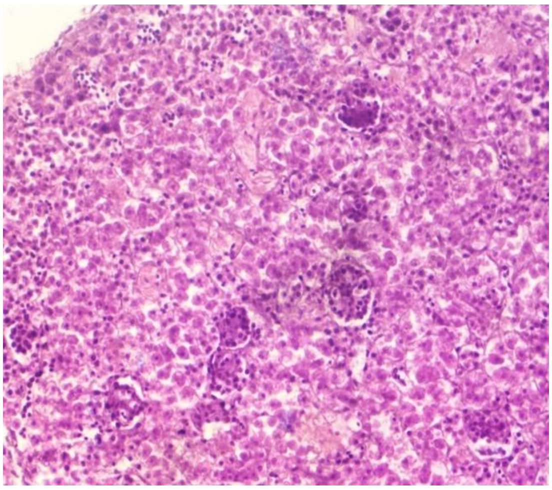


Fig. 62: Mild cellular degeneration, mild to moderate tubular degeneration in kidney

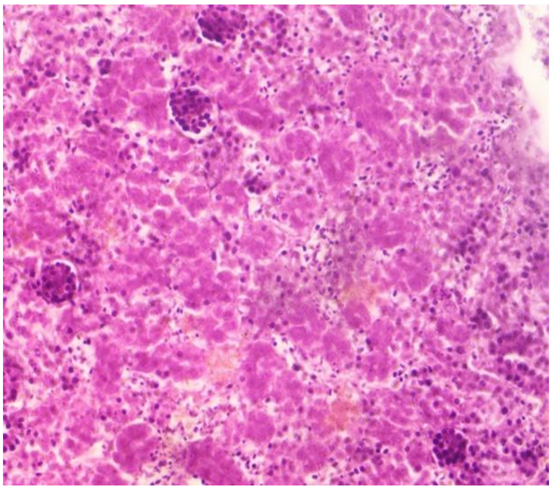


Fig. 63: Minimal infiltrations of inflammatory cells in kidney

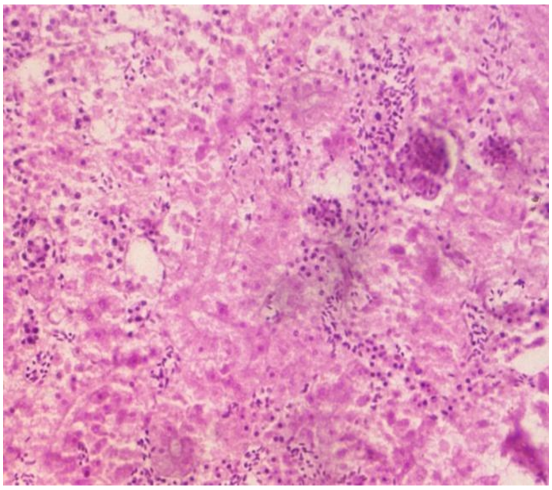


Fig. 64: Narrowing of the tubular lumen with congestion

DISCUSSION

Kidney is the major organ for ochratoxins, however it can also damage the liver, intestinal system, lymphoid tissue, skeletal system, hematopoietic tissues and reproductive system (Burns & Dwivedi, 1986). Furthermore It was observed that OTA residues are found in birds tissues and eggs (Iqbal *et al*., 2014)

5.1 Effect on Hatchability

After completion of incubation period, maximum hatchability was seen in control group (G-1) followed by Trp group (G-2) and than OTA+Trp (G-4) group while least hatchability was seen in OTA treated groups (G-3). The percentage of hatchability in different groups (G-1, G-2, G-3 and G-4) are (70, 66, 20 and 40) respectively. Similarly, Bryla *et al.* (2002) found that the reduced hatchability in OTA treated groups was observed due to any potential metabolite of OTA and accumulation of OTA in liver and yolk sac. Major mortality in growing chick embryos within OTA infected eggs happens at early developmental stages which may be due to teratogenic effects of OTA particularly mandibular hypoplasia, maxillary retrognathism and everted visera, which interfere with the hatching processes in fully grown chick embryos (Celik *et al*., 2000). Defective or incomplete closure may be due to cell death in the ventral part of the neural tube in OTA-treated chicken embryos (Wei and Sulik 1996). Reduced hatchability may be due to DNA adduct formation and subsequently inhibition of protein synthesis (Petkova-Bocharova *et al.,* 2003). The increase of OTA in feed for reproductive hens, reduced the level of fertilized egg hatchability due to increased embryo mortality at various stages of development (Niemiec *et al.*, 1994).

5.2 Effect on body weight

On 0^{th} day of hatching it was observed that there was a significant difference in body weight of ducklings in OTA treated group (G-3) in comparison to control (G-1) and Trp group (G-2). Group 2 containing Trp has shown highest body weight whereas 3^{rd} group containing OTA has lowest body weight of ducklings. On completion of 4^{th} week, there was significant difference ($P<0.01$) in body weight in OTA treated group (G-3) in comparison to control group (G-1) and Trp group (G-2). However, body weight in OTA+Trp group (G-4) is higher than OTA group (G-3) which shows in-ovo

Tryptophan improves growth performance and has ameliorative effect over OTA infected birds. Similarly Hassan *et al*. (2012) also noticed that chicks born from OTA-contaminated eggs have noticeably lighter body weights than their control counterpart chicks. This reduction in body weight is related to the harmful effects of OTA, particularly a decline in protein synthesis, which is essential for foetal development. The decreased body weights in OTA-fed groups may be due to impaired protein metabolism. The OTA competes with phe (phenylalanine) for binding sites on the Phe-transfer RNA synthetase enzyme, thus inhibiting protein synthesis and hence body growth (Creppy *et al.*, 1980). As per Zhai and Zhu 2021 findings OTA can alter the digestive and absorptive processes in ducks, which is considered for reduced weight of birds. Similarly Wyatt *et al.* (1981) observed that OTA toxicity affects growth rate in a dose dependent manner.

5.3 Effect of Tryptophan on Hb, PCV, TEC in ducklings which are exposed to OTA

Ducklings hatched out from OTA inoculated eggs showed least values of Hb, PCV, TEC, in comparison to other groups. Similar results of decreased RBCs count in response to OTA feeding has been reported by other researchers (Mohiuddin *et al*., 1993; Stoev *et al*., 2011) Elaroussi *et al*. (2006) observed that OTA intoxicated broiler birds showed decreased values of RBCs, PCV and Hb. Agag (2004) observed that ochratoxicosis may cause the decreases in RBC and Hb content as a result of lower serum iron levels and also causes anaemia.

5.4 Effect of Tryptophan on Total Leukocyte Count (TLC), Differential Leuckocyte Count (DLC) in ducklings which are exposed to OTA

There was no significant difference observed between the values of heterophils, basophils, eosinophils and lymphocytes in all 4 groups, although there was a significant difference ($P<0.01$) found in the value of monocytes which was higher in OTA treated group (G-3) as compared to control group (G-1) and Trp group (G-2). The TLC value of OTA treated group was significantly lower than control group (G-1) whereas TLC value of Trp group (G-2) was higher among all the groups. There was no significant difference is seen between the TLC values of OTA (G-3) group and OTA+T group (G-4). Similar to our findings Tansakul *et al*. (2012) observed that Total White Blood Cell

(WBC) Count decreases in OTA-Treated group as OTA contamination has a tendency to suppress certain immune cells in duck even at low levels of exposure. Chang *et al*. (1981) observed that ochratoxicosis causes drop in total circulating WBCs, which was attributed to a reduction in lymphocytes and monocytes. Zahoor-ul-Hassan *et al*. (2011) observed that OTA may have degenerative or necrotic effects on the follicles of immunological organs of chicks fed OTA-contaminated feed and hence causes atrophy of Bursa and normal formation of blood cells.

5.5 Effect on biochemical parameters

There was no significant difference in Glucose, Cholesterol and Triglyceride level in all the 4 groups, which shows that values of Glucose, Cholesterol and Triglyceride was not affected by OTA as well as Trp treatment. SGOT and SGPT of OTA treated group was found significantly higher than control and Trp group. The increase in ALT (SGPT) and AST (SGOT) level is a sensitive indicator of hepatotoxicity (Hassan *et al.*, 2010 and Hassan *et al.*, 2011). Similar increasing trend in ALT values in OTA treated birds was reported by several researchers, Sawale *et al*. (2009), Wang *et al.* (2009) which showed that OTA has potent hepatotoxic effect and it also causes acute hepatic necrosis.

The total protein and globulin in OTA inoculated group is significantly ($P<0.05$) lower than that of control (G-1) and Trp group (G-2). Similarly (Hassan *et al*., 2010) observed that total protein decreased in a dose related manner and was minimum in OTA treated group. Similar to our findings Kalorey *et al.* (2005) observed that Ochratoxin A causes significant reduction in serum proteins in OTA treated group. The present finding is in agreement with Manning and Wyatt (1984), Ramadevi *et al*. (2000) and Stoev *et al*. (2000).

The values of serum BUN, creatinine and uric acid was higher in OTA inoculated group (G-3) ducklings in comparison tocontrol group (G-1) and Trp group (G-2). Similarly Stoev *et al*. (2000) observed that elevated serum BUN, creatinine and uric acid were the indicators of nephrotoxic effect of OTA in ducklings. Our findings of increased creatinine values in OTA treated groups coincide with the results of other researchers (Kalorey *et al*., 2005; Sawale *et al.*, 2009 and Elaroussi *et al*., 2008). Increased serum creatinine levels reflects OTA induced nephropathy and impaired renal function (Garcia *et al*., 2003; Sakhare *et al.*, 2007; Elaroussi *et al*., 2008).

There was a significant elevation in serum uric acid level in OTA treated group was also evident by studies of Manning and Wyatt (1984) and Ramadevi *et al*. (2000) indicating that there was inhibition of protein accretion due to OTA. The results indicated impaired renal functions due to ochratoxicosis as evidenced by elevated serum uric acid in broilers. Stoev *et al*. (2000); Elaroussi *et al*. 2008 also reported elevated serum uric acid levels in broilers due to feeding of OTA. Similar to our findings (Stoev *et al*., 2002; Koynarski *et al*., 2007; Denli *et al*., 2008) also reported high urea (BUN) concentration in OTA fed chickens.

The serum levels of BUN, Creatinine and uric acid in OTA+T group (G-4) group is lower than OTA group (G-3) which shows ameliorative effect of Tryptophan over Ochratoxin infected ducklings.

5.6 Histopathology

On inoculating the duck embryo with ochratoxins on 0^{th} day and observing the changes on 29^{th} day, the major histopathological changes were noticed predominantly in liver and kidney. The hepatic parenchyma was infiltrated with mononuclear cells, similar findings were reported by Kumar *et al*. (2004). The hepatocytes were necrosed along with the evidence of oedamatic fluid. The architectural details of the liver were distorted along with the evidence of few pyknotic cells. The above findings were in accordance to the studies of Manimaran *et al*. (2003) and Singh *et al*. (1993) respectively. Similar to the hepatic parenchyma, subcapsular infiltrations of the mononuclear cells were also evident in the kidney along with necrosis in the renal epithelium. Similar observations were reported by the histopathological studies conducted by Mohiuddin *et al*. (1992), Kubena *et al*. (1985) and Huff *et al*. (1974). Some other notable histopathological alterations observed in kidneys include necrosis of renal tubular epithelial cells, disorganization and / or dissolution of renal tubular epithelial lining cells along with congestion and narrowing of renal tubular epithelium. The histopathological studies of the major lymphoid organs namely Bursa of Fabricus and thymus revealed depletion of bursal and thymic follicles. By the above results it can be concluded that ochratoxin is producing more alterations in kidney than liver or any other organ thus it is more nephrotoxic than hepatotoxic. Similar inferences were drawn by Kumar *et al*. (2004) in their study.

SUMMARY AND CONCLUSION

The present study was designed to evaluate the Ochratoxin-A induced toxicity on hatchability, body weight, haematological, biochemical parameters along with gross and histopathological changes in ducklings and ameliorative effect of L-Tryptophan in the Ochratoxin-A infected eggs of white pekin ducks.

The reduced hatchabilityupto 20% was noticed in Ochratoxin-A@50ng exposed eggs in comparison to healthy eggs. L-Tryptophan has significantly improved the hatchability percentage in Ochratoxin-A exposed eggs in comparison to only Ochratoxin-A exposed eggs. L-Tryptophan has positive effect in enhancing the hatchability percentage in normal healthy eggs.

From day 0 to 4 weeks of age, least body weight was observed in ducklings which are hatched from the Ochratoxin-A inoculated eggs in comparison to healthy eggs. L-Tryptophan has significantly improved the body weight of Ochratoxin-A inoculated hatched ducklings in comparison to only ochratoxin inoculated hatched ducklings. L-Tryptophan inoculated hatched ducklings showed maximum body weight which signifies that L-Tryptophan promotes body growth and development.

Ducklings hatched out from Ochratoxin-A intoxicated eggs showed disturbance in haematopoesis which is evident by least serum values of Hb, PCV, TEC and TLC in comparison to ducklings of other groups. L-Tryptophan treated ducklings showed good improvement in the serum levels Hb, PCV, TEC and TLC in Ochratoxin-A intoxicated ducklings. Maximum values of Hb, PCV, TEC and TLC was noticed in L-Tryptophan treated ducklings. Ducklings hatched out from Ochratoxin-A intoxicated eggs showed increased monocytes in their blood serum in comparison to non toxicated birds. L-Tryptophan treated ducklings showed decreased monocytes in Ochratoxin-A intoxicated ducklings.

Ducklings hatched out from Ochratoxin-A intoxicated eggs showed highest serum values of BUN, Creatinine, Uric Acid, ALT and AST in comparison to ducklings of other groups which sinifies that Ochratoxin-A is nephrotoxic and damages the kidney. Ducklings hatched out from Ochratoxin-A intoxicated eggs showed highest serum values of ALT and AST in comparison to ducklings of other groups which sinifies that Ochratoxin-A is hepatotoxic and harmful effects on liver. L-Tryptophan treated ducklings showed decrease in the serum levels of BUN, Creatinine, Uric Acid, ALT and AST in Ochratoxin-A intoxicated ducklings. Ducklings hatched out from Ochratoxin-A intoxicated eggs showed least serum values of Total Protein and globulin in their blood serum in comparison to non toxicated birds.

On pathological analysis, both gross as well as microscopic lesions are more evident in Ochratoxin-A treated groups. Grossly, hydrocephalus, teratogenic effect on embryonic growth, enlarged congested liver with petechial haemorrhages, enlarged kidney with congestion, atrophy of thymus and Bursa is seen in Ochratoxin-A inoculated embryos. L-Tryptophan treatment in Ochratoxin-A inoculated embryos showed less abnormal growth, mild congestions of liver and kidney, slightly enlarged liver and kidney. Only L-Tryptophan treated embryos do not showed any gross pathological alteration in body, head, and organs like liver, kidney, thymus and bursa.

Microscopical lesions in OTA treated group embryos revealed disorganization, degeneration and necrosis of hepatocytes, vacuolar degeneration and congestion of hepatocytes, infiltration of inflammatory cells and edema fluids in hepatocytes and tubular cells, tubular necrosis, narrowing of tubular lumen, dissolution of renal tubular epithelial lining cells and depletion of thymic follicles. L-Tryptophan treatment in Ochratoxin-A inoculated embryos showed mild to moderate histopathological alterations like minimal congestion and less infiltration of mononuclear cells, mild vacuolar degeneration, minimal dissolution of tubular epithelial cells in comparison to Ochratoxin-A treated group. L-Tryptophan treated embryos do not showed any noticeable histopathological alteration except infiltration of mononuclear cells and vacuolar degeneration of hepatocytes.

CONCLUSION

- *In ovo* Ochratoxin exposure cause reduced duck productivity and growth thus affects overall performance. OTA also perturbed blood and biochemical parameters as well as caused histopathological changes.
- In the current investigation, it was observed that Tryptophan had ameliorating effects @ 5mg/egg in reducing the negative impacts of ochratoxin in ducks.

- As a result, the current study found that incorporating L-tryptophan in ochratoxin contaminated diets could be helpful in mitigating ochratoxin's detrimental effects in ducks, such as growth abnormalities, haemato-biochemical alterations, and gross and histologapathological changes.

REFERENCES

Adeola. 1998. Bioavailability of tryptophan in soybean meal and tryptophan retention in the carcasses of four-week-old ducks. *Poultry science*, **77 (9):** 1312-1319.

Agarwal P, Singh D, Raisuddin S, Kumar R. 2020. Amelioration of ochratoxin-A induced cytotoxicity by prophylactic treatment of N-Acetyl-L-Tryptophan in human embryonic kidney cells, *Toxicology* , **429:** 152324.

Bailey, C. A, Gibson RM, Kubena LF, Huff WE and Harvey RB. 1989. Ochratoxin A and Dietary Protein- Effects on Hematology and Various Clinical Chemistry Measurements, *Poultry science*, **68(12):** 1664-1671.

Baliarsingh SK, Rao AG and Mishra PR. 1993. Pathology of experimental colibacillosis in chicks, *Indian Veterinary Journal* , **70:** 808 /812.

Battacone G, Nudda A, and Pulina, G. 2010. Effects of ochratoxin A on livestock production, Toxins, **2(7)**: 1796-1824.

Bennett J and Klich M. 2003. Mycotoxins, *Clinical microbiology reviews*, **16(3)**: 497-516.

Bhanja SK and Mandal AB. 2005. Effect of in ovo injection of critical amino acids on pre-and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens, *Asian-australasian journal of animal sciences*, **18(4):** 524-531.

Bharathi R, Pazhanivel N, Balachandran C, and Raj GD. 2014. Effect of dietary ochratoxin on biochemical and antioxidant profile in broiler chickens. *Indian Journal of Poultry Science*, **49(3):** 331-333.

Bhatti SA, Khan MZ, Saleemi MK and Hassan ZU. 2019. Impact of dietary Trichosporon mycotoxinivorans on ochratoxin A induced immunotoxicity-In vivo study, *Food and Chemical Toxicology*, **132:** 110696.

Brown MH, Szczech GM, Purmalis BP. 1976. Teratogenic and toxic effects of ochratoxin A in rats. *Toxicology and Applied Pharmacology*, **37(2):** 331-8.

Bryła M, Damaziak K, Twarużek M, Waśkiewicz A, Stępień L, Roszko M and Wójcik W. 2022. Toxico-pathological effects of ochratoxin A and its diastereoisomer under in ovo conditions and in vitro evaluation of the toxicity of these toxins against the embryo Gallus gallus fibroblast cell line, *Poultry Science*, 102413.

Burditt SJ, Haggler WM Jr and Hamilton PB. 1984. Feed refusal during ochratoxicosis in turkeys, *Poultry Science,* **63(11):** 2172-2174.

Chang CF, Huff WE and Hamilton PB. 1979. A leucocytopenia induced in chickens by dietary ochratoxin A, *Poultry science*, **58(3):** 555-558.

Corrier DE. 1991. Mycotoxicosis- mechanisms of immunosuppression. *Veterinary immunology and immunopathology*, **30(1):** 73-87.

Council for Agricultural Science. 2003. Mycotoxins: risks in plant, animal, and human systems *Council for Agricultural sciences*, **139.**

Davies MJ, Hawkins CL, Pattison DL, and Rees MD. 2008. Mammalian heme peroxidases- from molecular mechanisms to health implications, *Antioxidants and redox signaling*, **10(7):** 1199-1234.

Denli M, Blandon JC, Guynot ME, Salado S and Perez JF. 2008. Efficacy of a new ochratoxin-binding agent (OcraTox) to counteract the deleterious effects of ochratoxin A in laying hens, *Poultry Science*, **87(11):** 2266-2272.

Dwivedi P and Burns RB. 1984a. Pathology of ochratoxicosis in young broiler chicks, *Research in Veterinary Science*, **36:** 92 103.

Dwivedi P and Burns RB. 1986. The natural occurrence of ochratoxin A and its effects in poultry A review: Part I. Epidemiology and toxicity, *World's Poultry Science Journal*, ***42*** **(1):** 32-47.

Elaroussi MA, Mohamed FR, Barkouky EM, Atta AM, Abdou AM and Hatab MH. 2008. Experimental ochratoxicosis in broiler chickens, *Avian Pathology,* **35:** 263-269.

Frye CE and Chu FS 1977. Distribution of ochratoxin A in chicken tissues and eggs, *Journal of food safety*, **1(2):** 147-159.

Garcia AR, Avila E, Rosiles R, and Petrone VM 2003. Evaluation of two mycotoxin binders to reduce toxicity of broiler diets containing ochratoxin A and T-2 toxin contaminated grain, *Avian diseases*, **47(3):** 691-699.

Gibson RM, Bailey CA, Kubena LF, Huff WE, Harvey RB. 1990. Impact of L-phenylalanine supplementation on the performance of three-week-old broilers fed diets containing ochratoxin A. 1. Effect on body weight, feed conversion, relative organ weight, and mortality, *Poultry Science,* **69:** 414–419.

H and O'Brien KK. 2010. Scoping studies-advancing the methodology. *Implementation science*, **5(1):** 1-9.

Hameed MR, Khan MZ, Saleemi MK, Khan A, Akhtar M, Hassan ZU, and Hussain Z. 2017. Study of ochratoxin A (OTA)-induced oxidative stress markers in broiler chicks, *Toxin Reviews*, **36(4):** 270-274.

Hassan ZU, Khan MZ, Khan A, Javed I, and Hussain Z. (2012). Effects of individual and combined administration of ochratoxin A and aflatoxin B1 in tissues and eggs of White Leghorn breeder hens, *Journal of the Science of Food and Agriculture*, **92(7):** 1540-1544.

Hassan ZU, Khan MZ, Khan A, Javed I, Sadique U and Khatoon A. 2012. Ochratoxicosis in White Leghorn breeder hens Production and breeding performance, Pakistan Veterinary Journal, **32(4):** 557-561.

Haubeck HD, Lorkowski G, Kölsch E, and Röschenthaler R. 1981. Immunosuppression by ochratoxin A and its prevention by phenylalanine, *Applied and Environmental Microbiology*, **41(4):** 1040-1042.

Hoehler D and Marquardt RR. 1996. Influence of vitamins E and C on the toxic effects of ochratoxin A and T-2 toxin in chicks, *Poultry science*, **75(12):** 1508-1515.

Huff W and Hamilton P. 1979. Mycotoxins-their biosynthesis in fungi: Ochratoxins-metabolites of combined pathways, *Journal of Food Protein*, **42:** 815–820.

Huff WE, Kubena LF and Harvey RB. 1988. Progression of ochratoxicosis in broiler chickens, *Poultry science*, **67(8):** 1139-1146.

Huff WE, Wyatt RD, Tucker TL, and Hamilton PB. 1974. Ochratoxicosis in broiler chickens, Poultry Science, **53:** 1585 /1591.

Jelinek CF, Pohland AE and Wood GE 1989. Worldwide occurrence of mycotoxins in foods and feeds—an update. *Journal of the Association of Official Analytical Chemists,* **72(2):** 223-230.

Kadakal C, Batu HS, Tepe TK Sengun P and Otag FB. 2018. Which one may be the best quality parameter of risky foods in terms of mold-aflatoxins, patulin, ochratoxin a and ergosterol, *Agriculture and Food*.

Kalorey DR, Kurkure NV, Ramgaonkar JS, Sakhare PS, Warke PS, and Nigot NK. 2005. Effect of polyherbal feed supplement, *Asian-Australasian journal of animal sciences*, **18(3):** 375-383.

Khatoon A, Abidin Z. 2018. An extensive review of experimental ochratoxicosis in poultry. I. Growth and production parameters along with histopathological alterations. *World Poultry Science Journal* , **74:** 627–646.

Khatoon A, Zargham Khan M, Khan A, Saleemi MK, and Javed I. 2013. Amelioration of Ochratoxin A-induced immunotoxic effects by silymarin and vitamin E in White Leghorn cockerels, *Journal of immunotoxicology,* **10(1):** 25-31.

Kubena LF, Harvey RB, Fletcher OJ, Phillips TD, Mollenhauer DA, Witzell DA and Heidelbaugh ND. 1985. Toxicity of ochratoxin A and vanadium on growing chicks, *Poultry Science,* **64:** 620 /628

Kubena LF, Harvey RB,Huff WE, Corrier DE, Phillips DE, and Rottinghaus GE. 1989. Influence of ochratoxin A and T-2 toxin singly and in combination on broiler chickens, Poultry Science, **68:** 867-872.

Kumar A, Jindal N, Shukla CL, Asrani RK, Ledoux DR, and Rottinghaus GE. 2004. Pathological changes in broiler chickens fed ochratoxin A and inoculated with Escherichia coli, *Avian Pathology*, **33(4):** 413-417.

Kumar R, Ansari KM, Saxena N, Dwivedi PD, Jain SK and Das M. 2012. Detection of ochratoxin A in wheat samples in different regions of India. *Food control,* **26(1):** 63-67.

Liu DW, Liu HY, Zhang HB, Cao MC, Sun Y, Wu WD, and Lu CH. 2016. Potential natural exposure of endangered red-crowned crane (Grus japonensis) to mycotoxins aflatoxin B1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A, *Journal of Zhejiang University-science B*, **17(2):** 158-168.

Liuzzi VC, Fanelli F, Tristezza M, Haidukowski M, Picardi E, Manzari C and Mule G. 2017. Transcriptional analysis of Acinetobacter sp. neg1 capable of degrading ochratoxin A. *Frontiers in microbiology*, **7:** 2162.

Malir F, Ostry V and Novotna E. 2013. Toxicity of the mycotoxin ochratoxin A in the light of recent data, *Toxin Reviews*, **32(2):** 19-33

Mally A, Pepe G, Ravoori S, Fiore M, Gupta RC, Dekant W and Mosesso P. 2005. Ochratoxin A causes DNA damage and cytogenetic effects but no DNA adducts in rats, *Chemical research in toxicology*, **18(8):** 1253-1261.

Manimaran K, Singh SD and Shivachandra SB. 2003. Haematobiochemical and pathological changes in experimental Escherichia coli infection in broiler chicks, Indian Journal of Animal Sciences, **73:** 960 /962

Marquardt RR and Frohlich AA. 1992. A review of recent advances in understanding ochratoxicosis, Journal of Animal Sciences, **70:** 3968–3988.

Micco C, Miraglia M, Brera C, Corneli S and Ambruzzi A 1995. Evaluation of ochratoxin A level in human milk in Italy, *Food Additives and Contaminants*, **12(3):** 351-354.

Mohiuddin SM, Reddy MV and Ahmed SR. 1992. Studies on ochratoxicosis in broiler chicks, Indian Veterinary Journal , **69:** 1011 / 1014.

Nayak N, Rajini RA, Kirubaharan JJ, Ezhilvalavan S and Sahu AR. 2018. Effect of in ovo feeding of tryptophan on post-hatch production performance and immune response in commercial broilers. *Animal Nutrition and Feed Technology* , **18(3):** 355-366.

Niemiec J, Borzemsk W, Golinski P, Karpinska E, Szeleszczuk P, and Celeda T. 1994. The effect of ochratoxin A on egg quality, development of embryos and the level of toxin in eggs and tissues of hens and chicks, *Journal of Animal Feed Science*, **3(4):** 309-316.

Nowaczewski S, and Kontecka H. 2012. Haematological indices, size of erythrocytes and haemoglobin saturation in broiler chickens kept in commercial conditions, *Animal Science Papers and Reports*, **30(2):**

O'Brien E, Heussner AH and Dietrich D. 2001. Species- sex- and cell type specific effects of ochratoxin A and B, *Toxicological Sciences*, **63:** 256.

Petkova-Bocharova T, Castegnaro M, Pfohl-Leszkowicz A, Garren L, Grosso F, Nikolov I and Chernozemsky IN. 2003. Analysis of ochratoxin A in serum and urine of inhabitants from an area with Balkan Endemic Nephropathy: A one month follow up study, The Facta Universitatis, Series: Medicine and Biology, **10:** 62-68.

Pfohl-Leszkowicz A, Petkova-Bocharova T, Chernozemsky IN, Castegnaro MB. 2002. Endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins, *Food Additives and Contaminants*, **19:** 282–302.

Prior MG, O'neil JB and Sisodia CS. 1980. Effects of ochratoxin A on growth response and residues in broilers, *Poultry science*, **59(6):** 1254-1257

Rached E, Pfeiffer E, Dekant W, Mally A. 2006. Ochratoxin A: Apoptosis and aberrant exit from mitosis due to perturbation of microtubule dynamics, *Toxicological Sciences*, **92:** 78–86.

Raju MVLN and Devegowda G. 2000. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin), *British poultry science*, **41(5):** 640-650. Sci. 1979, 59, 605–609.

Ribelin WE, Fukushima K and Still PE. 1978. The toxicity of ochratoxin to ruminants, *Canadian Journal of Comparative Medicine*, **42(2):** 172.

Ricci FG, Terkelli LR, Venancio EJ, Justino L, Dos Santos BQ, Baptista AAS and Itano EN. 2020. Tryptophan attenuates the effects of OTA on intestinal morphology and local IgA/IgY production in broiler chicks, *Toxins*, **13(1):** 5.

Ringot D, Chango A, Schneider YJ and Larondelle Y. 2006. Toxicokinetics and toxicodynamics of ochratoxin A, *Chemico-biological interactions*, **159(1):** 18-46.

Santin E, Paulillo AC, Maiorka PC, Alessi AC, Krabbe EL, and Maiorka A. 2002. The effects of ochratoxin/aluminosilicate interaction on the tissues and humoral immune response of broilers, *Avian Pathology*, **31(1):** 73-79.

Sawarkar AR, Sonkusale PM, Kurkure NV, Jangade CR, Maini S and Ravikant K. 2011. Experimental afla and ochratoxin induced mixed mycotoxicosis in broilers and its amelioration with herbomineral toxin binder Toxiroak Gold. *International Journal of Poultry Science.*

Scudamore KA. 2000. Mycotoxins in stored grain, In *HGCA conference, Crop management into the Millenium. London,* (Vol. 6, pp. 18-1).

Stoev SD. 2010. Studies on carcinogenic and toxic effects of ochratoxin A in chicks, Special issue “Ochratoxins”, *Toxins*, **2:** 649–664.

Tansakul N, Kusujarit N, Kasorndorkbua C, Witoonsatian K and Songserm T. 2012. The effect of low-dose ochratoxin: A fed in ducks on blood haematological profiles and histopathological alterations, *Asian Journal of Animal and Veterinary Advances,* **7(10):** 1021-1027.

Van der Merwe K. J, Steyn PS, Fourie L, Scott DB and Theron JJ. 1965. Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus, *Wilh Nature*, **205(4976):** 1112-1113.

Vartiainen S, Yiannikouris A, Apajalahti J and Moran CA 2020. Comprehensive evaluation of the efficiency of yeast cell wall extract to adsorb ochratoxin A and mitigate accumulation of the toxin in broiler chickens, *Toxins*, **12(1):** 37.

Verma J, Johri TS and Swain BK. 2003. Effect of varying levels of aflatoxin, ochratoxin and their combinations on the performance and egg quality characteristics in laying hens, *Asian-australasian journal of animal sciences*, **16(7):** 1015-1019.

Vesala, D, Vesely D, and Jelinek R. 1983. Toxic effects of ochratoxin A and Citrinin, alone and in combination, on chicken embryos, *Applied and Environment Microbiology*, **45:** 91–3.

Wangikar P, Dwivedi P, Sinha N, Sharma AK and Telang AG. 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects, *Toxicology*, **215:** (1-2), 37-47.

Wei X and Sulik KK. 1996. Pathogenesis of caudal dysgenesis/sirenomelia induced by ochratoxin A in chick embryos, *Teratology*, **53(6):** 378-391.

Zahoor-ul-Hassan H, Muhammad Zargham K, Ahrar K, Ijaz J and Mnaza, N. 2012. In vivo and ex vivo phagocytic potential of macrophages from progeny of breeder hens kept on ochratoxin A (OTA)-contaminated diet, *Journal Immunotoxicology*, **9:** 64-71.

Zahoor-ul-Hassan H, Muhammad Zargham K, Ahrar K, Ijaz J and Mnaza N. 2012. In vivo and ex vivo phagocytic potential of macrophages from progeny of breeder hens kept on ochratoxin A (OTA)-contaminated diet, *Journal of Immunotoxicology*, **9:** 64-71.

Zhai S, Zhu Y, Feng P, Li M, Wang, W, Yang L, and Yang Y. 2021. Ochratoxin A: Its impact on poultry gut health and microbiota, an Overview, *Poultry science*, **100(5):** 101037.