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The Effect of Organophosphate Insecticides in Cattle Farming Feed on FSH and LH Hormone Levels in Dairy Cows in Kediri and Pujon

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Abstract: The issue plaguing the dairy cattle industry as a whole is low reproductive efficacy due to nutritional and reproductive disorders. To overcome this, nutrients in feed play a very important function. Feed is an essential component of any livestock operation. The requirements for animal protein depend on physiological status and production level. Ruminants can also reduce protein loss by recycling urea, a normally excreted byproduct of protein metabolism. Insecticides are components of pesticides used to kill insects. Organophosphate insecticides (OPs) are phosphate-containing insecticides. Phosphoric acid used to manufacture organophosphate insecticides. This insecticide is notorious for being the most toxic to mammals. This study seeks to determine the effect of feed-containing organophosphates on FSH and LH hormone concentrations in the blood of dairy cows. Mid-lactation For the purpose of this study, Friesian Holstein dairy cattle were divided into two experimental groups, group 1 (first ration) and group 2 (second ration). ANOVA analysis was used for statistical analysis.

Keywords: Friesian Holstein (FH); FSH; LH; Organophosphate; ROS

Introduction

Pesticides have a crucial role in several applications. The application of pesticides in the domains of agriculture and fisheries has been identified as advantageous in terms of augmenting productivity. The primary purpose of utilizing pest management strategies is to safeguard production outcomes from potential losses resulting from a diverse array of pests, encompassing pests, illnesses, and weeds (Bruinsma, 2017). Nevertheless, the utilization of this herbicide exerts adverse effects on human beings, the biota, and the surrounding ecosystem. The degradation of the aquatic environment is a detrimental consequence associated with the disposal of pesticide waste (Carvalho, 2017). The Diazinon pesticide, classified as an organophosphate insecticide, is extensively employed in agricultural industries for the purpose of eliminating troublesome pests (Freeman et al., 2005). The demand for this pesticide has been shown to consistently rise over time. Diazinon 60 EC is an extensively utilised pesticide belonging to the class of organophosphates (Faggio et al., 2018). The escalating utilization of this technology, if employed in an erroneous manner, will exert an adverse influence on diverse facets of existence, encompassing the issue of agricultural contamination (Kumar et al., 2013). The potential for residues in agricultural and livestock products arises from the overutilization of pesticides and non-compliance with the usage guidelines established by producers (Jones et al., 2015). The potential existence of pesticide residues in food items has the capacity to give rise to public health concerns among consumers, manifesting as instances of poisoning, immunosuppression, and potentially carcinogenic effects (Maurya & Malik, 2016).

Diazinone, also known as O,O-diethyl, is the subject of discussion. The chemical compound O-[6-methyl-2-(1-methylethyl)- The compound referred to as

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phosphorothioate or O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidyl) Phosphorothioate is the designated nomenclature for a synthetic pesticide classified within the organophosphate category, which obtained its initial registration in the United States in 1956 (Abbasian et al., 2014). The exposure to diazinon has been found to induce toxic effects by inhibiting the activity of acetylcholinesterase (AChE) in the nervous system (Laetz et al., 2013). This inhibition leads to the accumulation of acetylcholine (ACh) at synapses and neuromuscular junctions, resulting in adverse physiological consequences (Dzul-Caamal et al., 2014). Organophosphate poisoning exhibits a diverse range of symptoms associated with cholinergic crises and impairment of hepatic and renal function. The liver and kidneys are susceptible to the effects of diazinone due to their crucial roles in the metabolism and excretion of this compound (Flores et al., 2014). Multiple prior research have substantiated the detrimental effects of diazinon, which include inducing histopathological harm and disrupting metabolic equilibrium (Cai et al., 2013).

According to Karyab et al. (2013) and Geissen et al. (2015), the administration of diazinon at a dosage of 10 mg/kg body weight has been seen to result in an approximate 1.2-fold rise in Blood Urea Nitrogen (BUN) levels and a 1.1-fold increase in serum creatinine levels compared to the levels observed in normal control subjects. The exposure to Diazinon has been found to result in the formation of Reactive Oxygen Species (ROS) and a decrease in the activity of enzymes such as Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Glutathione Reductase (GR) (Boussabbeh et al., 2016). This enzymatic inhibition leads to the peroxidation of fats, as indicated by an observed elevation in Malondialdehyde (MDA) levels (Lari et al., 2014). In addition to the detrimental effects resulting from the formation of Reactive Oxygen Species (ROS), it has been observed that diazinon has the potential to induce DNA damage (Caixeta et al., 2012) and cellular damage via the apoptotic pathway (Fallah et al., 2020).

The reproductive system is cytotoxic and pathologically affected by diazinon (Abbasian et al., 2014). The aforementioned substance induces a diverse array of pathological consequences on the reproductive system, including but not limited to ovarian atrophy, disturbances in sex hormone regulation, impairments in oogenesis, diminished quality of ova, and challenges in achieving fertility (Ophir et al., 2014). Exposure to some agents has been found to result in many detrimental effects in animals, including ovum damage, inflammation, mitochondrial insufficiency, DNA fragmentation, apoptosis, and ultimately, cell death. The acronym "DZN" is a shorthand term commonly used in online communication (Karl et al., 2020). Reactive Oxygen Species (ROS)-induced Oxidative Stress (OS) has been identified as a potential primary mechanism related with ovum DNA fragmentation, apoptosis, antioxidant depletion, and subsequent compromised ovum quality and infertility in female animals (Montuori et al., 2016).

Method

Experimental Animal Preparation

The search conducted for six months on two farms located in the Kediri and Pujon. Blood samples were collected from the jugular vein, with a volume of 1-3cc. Prior to the collection of blood, an aseptic swab was used to cleanse the area with alcohol. The collected blood transferred into a serum separator tube and centrifugation at a speed of 67-3000 RPM for 15 minutes.

Follicle Stimulating Hormone (FSH) (cat number MBS590027) and Luteinizing Hormone (LH) (cat number MBS590027) was measured. First prepare reagents, samples, standard dilutions, and microplates for each preparation. Add 1 ml of deionized water to a standard, centrifuge, and allow stand for 5 minutes to prepare the standard preparation. To prepare the Kit Control, add 1.0 ml of deionized water and centrifuge. Prepare sample microplates and standard solutions. Next, add 50 µl of Assay diluents RD 1-63 to each well. Next, 50 µl of standard, control, and sample were added to each well and incubated at room temperature for 2 hours. While waiting for incubation, make 2 ml wash buffer from 1/25 x 50 ml. Washing was done 5 times after 2 hours of incubation. Following washing, 100 µl conjugate was added to each well and incubated for 2 hours at room temperature. Following incubation, washing was done 5 times. Add 100 µl of substrate after the second 2-hour incubation, and incubate for 30 minutes at room temperature, protected from light. Add 100 µl Stop Solution and mix after incubation. A 150nm ELISA Reader on 540/570 nm wavelenght (Zheng et al., 2016).

Data Analysis

The data analysis on FSH levels and the number of primary follicles conducted quantitatively. The quantitative data were analyzed statistically using the ANOVA test which has a 95% confidence level (α = 0.05) (Thomatou et al., 2013). The next analysis using the Honest Significant Difference (BNJ) or Tukey Test to find out the most significant different results.

Result and Discussion

ELISA Test Results of FSH Serum

The results of the statistical research indicate a correlation between Pujon and Kawi dairy cows. Based on the statistical findings, it can be shown that the data obtained from the study of Follicle-Stimulating Hormone (FSH) concentrations exhibited a normal distribution ($P > 0.05$) and heterogeneity (P 0.05), indicating significant variations among the data collected from the four groups. The findings from the analysis of variance (ANOVA) indicate that there are statistically significant differences in the means across the several groups (P 0.05). Therefore, it can be inferred that these differences are indeed significant.

Table 1. ELISA Serum of FSH

FSH Group	Mean ± SD (µg/100 ml)
Pujon	3.2725 ± 0.208
Kawi	0.7900 ± 0.200

The analysis's findings revealed that, in comparison to the Pujon cow group, the FSH levels in the Kawi region's cow group were generally lower. The presence of pesticide residue in the serum to distribution throughout various tissues inside the body, as well as the concentration of pesticides found in food and drinking water. These sources of pesticide contamination pose potential risks to the health of animals and the safety of their products. Organophosphate insecticides exhibit lipophilic properties, rendering them insoluble in water (Assidi et al., 2013). The majority of organophosphates exhibit limited metabolic activity within the human body, resulting in their accumulation within adipose tissue. Adiposa refers to a medical condition characterized by an excessive accumulation of body fat. As a result, these chemicals are frequently identified in adipose tissue and the hepatic organ. Both tissues are frequently employed as markers for the purpose of diagnosing animal toxicity. The toxicological impacts of organophosphate compounds manifest predominantly in a chronic manner, as opposed to an acute one, due to their inherent resistance to degradation within both the human body and the environment (Abbasian et al., 2014). Organophosphates have a propensity to interact with both motor and sensory nerve fibers, resulting in the manifestation of symptoms such as tiny muscular tremors (Amiri et al., 2018).

The introduction of organophosphate into the bovine organism through systemic means results in the impairment of the neurological system. This occurs as the organophosphate molecules engage in competitive binding with acetylcholine, a neurotransmitter, for the enzyme acetylcholinesterase. The process of acetylcholine hydrolysis, facilitated by the binding of the enzyme acetylcholinesterase, plays a crucial role in the termination of acetylcholine's effects. The inhibition of acetylcholinesterase enzyme activity leads to the buildup of acetylcholine at the synapses of cholinergic neuroeffectors, resulting in muscarinic effects. This phenomenon also occurs at the neuromuscular junctions and autonomic ganglia. The nicotine effect is the unhydrolyzed form of acetylcholine persists in binding to its receptors on the postsynaptic membrane, resulting in an extended period of vasoconstriction and detrimental impact on blood circulation (Derafshi et al., 2016).

The presence of organophosphate compounds in the bloodstream can lead to the inhibition of the neurological system responsible for regulating the buildup of acetylcholine at the synapses of cholinergic neuroeffectors. This will impede the secretion of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), as well as the female reproductive hormones circulated via the bloodstream. The reduction in Follicle-Stimulating Hormone (FSH) leads to ovarian atrophy and impairs the maturation of follicles, preventing them from progressing to the stage of ovulation (Palma et al., 2014).

The observed effect of the organophosphate chemical suggests that it hinders the functioning of the enzymes responsible for the deactivation of acetylcholine, inhibitory effects of organophosphates on pseudocholinesterases in plasma and cholinesterase in red blood cells and synapses attributed to the phosphorylation of anion ester components. The strength and irreversibility of these phosphorus bonds are highly pronounced. The inhibition of acetylcholinesterase persists until the synthesis of a new enzyme occurs or until the administration of a cholinesterase reactivator (Lewis et al., 2016).

The administration of organophosphate compounds observed to exert a suppressive effect on the Follicle-Stimulating Hormone (FSH) in the ovarian follicles. Consequently, this inhibition leads to a cessation of follicular development, ultimately culminating in either anovulation or degeneration of the follicles. The buildup of steroids in circulation, leading to congestion and bleeding, is a consequence of the disruption of steroid metabolism caused by organophosphate chemicals. Follicle cell degeneration is a consequence of hormonal imbalances. The maturation of follicle cells necessitates the presence of Follicle-Stimulating Hormone (FSH), while ovulation requires the release of Luteinizing Hormone (LH) by the pituitary gland. However, the accumulation of steroids in the bloodstream can inhibit the feedback response of the ovaries to the pituitary gland, resulting in a decrease in FSH levels and disruption of follicle maturation. This disruption accompanied by the degeneration of follicle cells (Storck et al., 2017).

Effect of Organophosphate Exposure on the LH Hormone in Dairy Cows

The statistical study reveals a noteworthy correlation between the Pujon and Kawi pear cows. Additionally, the statistical analysis demonstrates substantial associations between the Pear Cows and the Kawi. Based on the statistical findings, the examination data exhibits a normal distribution of concentrations (P > 0.05). However, the data is not homogenous (P < 0.05), indicating that the four groups differ from each other and are not identical. In order to satisfy the normality assumption for statistical testing, it is possible to proceed with Tukey tests following one-way ANOVA tests. The results of the ANOVA test indicate that there are statistically significant differences in the means of each group (P < 0.05), leading to the conclusion that the groups are considerably differ According to the analysis's findings, the Kawi region's cow group had higher amounts of LH hormone than the Pujon cow group had. The organophosphate group exerts harmful effects on feed by inhibiting the release of the enzyme esterase in the central nervous system. The organophosphate insecticide classified as a phosphoric acid ester or thiophosphate, and its toxicity linked to its capacity to hinder the function of acetylcholinesterase. This inhibition leads to the buildup of aceticylcholine at the nerve synapses.

Table 2. ELISA Serum of LH

LH Group	Mean ± SD (μg/100 ml)
Pujon	0.2735 ± 0.254
Kawi	0.9840 ± 0.384

According to Kaushik et al. (2012), in the event that the endocrine inhibitor or its metabolite forms a binding interaction with the estrogen receptor without inducing activation, it assumes an antagonistic role by deactivating the estrogen receptor and impeding the binding of estradiol. Consequently, the hypothalamus initiates the secretion of Gonadotropin-Releasing Hormone (GnRH), leading to an increased synthesis of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) by the pituitary gland. The concentration of estradiol will likewise experience an increase; however, because of the disruption of the feedback system, Gonadotropin-Releasing Hormone (gnRH) will not undergo a drop. In conclusion, prolonged exposure to elevated levels of estrogen and/or endocrine disruptors may lead to a decrease in the sensitivity of estrogen receptors. Furthermore, it is important to consider chronic exposures that may include immunotoxicity, carcinogenesis, endocrine alterations, and detrimental impacts on reproductive health.

According to Gaelle et al. (2014), Moreover, it disrupts the estrus cycle and diminishes follicular count through the augmentation of atretic follicle development, ovarian follicle death, and follicular depletion. Previous research conducted on several animal models has demonstrated that organophosphate pesticides have the ability to function as endocrine disruptors. In a study conducted by Yu et al. (2016), it revealed that there was a decline in serum Luteinizing Hormone (LH) levels following acute exposure to parathons. However, it shown that these levels were able to recover and return to baseline within a 24-hour period. Prolonged exposure to quinalphos at dosages below the fatal threshold leads to elevated levels of Luteinizing Hormone (LH), prolactin, and Follicle-Stimulating Hormone (FSH) in the serum of mice. The decline in Luteinizing Hormone (LH) concentrations linked to the presence of harmful organophosphate compounds. This decline primarily caused by the inhibitory effect of these compounds on acetylcholinesterase, an enzyme crucial for the breakdown of acetylcholine. Consequently, the inhibition of acetylcholinesterase disrupts the release of Gonadotropin-Releasing Hormone (GnRH) by modulating the activity of nicotine and muscarinic receptors. The impact of this substance on the nicotinic receptor leads to a transient release of Gonadotropin-Releasing Hormone (GnRH), while its impact on the muscarinic receptor hinders the release over extended durations. The ability of organophosphate to effectively inhibit estrogen-dependent tissue responses and induce anti-estrogenic effects, leading to ovarian dysfunction. Furthermore, the anti-estrogenic effects of organophosphate found to disrupt estrus cycles from each other.

Conclusion

The presence of hormonal imbalances in dairy cows suggests that their exposure to organophosphates has a detrimental effect on the endocrine function of the hypothalamus-pituitary axis, resulting in reduced levels of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Hormonal imbalances in dairy cattle identified by observing the levels of plasma Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). Notably, a distinct pattern emerges, wherein the FSH levels in the Pujon cattle group surpass those in the Kawi cattle group, while the LH levels in the Kawi cattle group exceed those in the Pujon cattle group.

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