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Research Article

Effects of Dietary Exposure of Rabbit Does to Di-(2-Ethylhexyl) Phthalate on Growth Performance, Blood Profile, Ovarian Follicles and Metric Parameters of Kittens

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Abstract

This study examined the systemic and cross-generational toxicological impacts of exposure to Di-(2-ethylhexyl) phthalate (DEHP) in female rabbits. Forty-five rabbit does were randomly distributed to five different groups with the following varied dietary inclusion levels of DEHP: 0 ppm (control), 100 ppm, 200 ppm, 300 ppm and 400 ppm, representing T1, T2, T3, T4 and T5 respectively. The daily feed intake remained similar ($p>0.05$), however, final live weight significantly ($p<0.05$) reduced at higher inclusion levels. Haematological analysis revealed a non-significant decrease in packed cell volume (PCV) and hemoglobin. Serum biochemical results showed an acute hepatotoxicity, typified by an almost three-fold rise in Aspartate animotranferase (AST) (from 33.65 in T1 to 99.47 IU/L in T5) and a statistically significant increase in Alanine aminotransferase (ALT) in the treatment groups. Endocrine disrupting property of DEHP was noticed by a significant drop of Luteinizing Hormone (LH) and Estradiol ($p < 0.05$), which shows a correlation with ovarian atrophy and a significant erosion of Graafianfollicles. Furthermore, neonatal indices indicated serious developmental retardation as evidenced by the decreased anogenital distance and crown-rump length at T1 and T2, alongside absolute reproductive failure at 300 and 400 ppm inclusion levels. It can be concluded from this study that dietary DEHP is an endocrine disruptor in rabbits, with a potential to compromise metabolic health of rabbits, leading to total infertility at high doses.

Keywords

Di-(2-Ethylhexyl) Phthalate, Rabbit Does, Ovarian Follicles, Gonadotrophins, Blood Profile, Hormone Synthesis, Reproductive Toxicity

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1. Introduction

The swift rate of industrialization that is witnessed in the world economy has resulted in rapid spread in the use of certain synthetic chemicals which most times get into the agricultural value chain. One of such chemicals is phthalate also known as phthalate acid esters. They are compounds synthesized from long-chain alcohols [1]. At ambient temperature, they exist as liquids that are tasteless and nonirritating [2], exhibiting low solubility in water while being more soluble in fats [3]. These compounds are utilized in manufacture of plastics to impart flexibility and to enhance their durability. Notably, phthalate plasticizers are not covalently bonded to polyvinyl chloride, which allows for the potential leaching, migration, or evaporation into the environment, food and animal feed components [4, 5]. This characteristic facilitates their entry into the body via inhalation, dermal absorption, or through the bloodstream [6].

DEHP is toxicologically categorized as an endocrine-disrupting chemical and its metabolites, particularly Mono-(2-ethylhexyl) phthalate (MEHP), obstruct the endocrine system by imitating and stopping natural hormones [7]. One of the targets of DEHP is the Hypothalamic-Pituitary-Gonadal (HPG) axis where it interferes with the much required hormonal signaling for follicular development and pregnancy. Animal studies have demonstrated that the epithelial cells of the digestive tract can absorb phthalates, and it has been observed that the metabolites produced post-absorption tend to be more toxic [8]. When DEHP is administered orally, it can be hydrolyzed by pancreatic lipases in the small intestine, resulting in the formation of MEHP (monoethyl hexyl phthalate) or 2-ethylhexanol phthalate, which subsequently enters the systemic circulation. Research has also indicated that absorption rates may vary among species, with younger animals exhibiting more frequent intestinal absorption compared to adults [9].

Phthalates are now considered acutely toxic, and data regarding chronic effects is primarily derived from studies conducted on experimental animals [10]. Rabbits usually play a role as models for investigating toxicological impacts of chemical particularly as it affects the reproduction and maternal-fetal linkage [11]. This is so because rabbits are induced ovulators making it possible to examine follicular activities under a controlled environment. The first impact of DEHP exposure is usually observed in growth performance, as it has the potential to interlope the pathways of metabolism and thyroid axis, thereby causing a decline in the feed intake as well as poor absorption of feed nutrients and reduced weight gain in does [12, 13]. These deleterious effects are dangerous for the development of fetuses and lactation following kindling [13].

Furthermore, exposure to DEHP has been proven to possess the tendency to induce oxidative stress in human model [14]. There have been reported reductions in red blood cells, white blood cells, platelets, albumin, alkaline phosphatase (ALP) [15], leukocytes and lymphocytes [16] while significant increases in creatinine and aspartate aminotransferase (AST) were observed by [15]. DEHP has been implicated for triggering follicular atresia at the ovary, causing the necrosis of ovarian follicles, thus leading to notable decline in the number of active follicles ready for ovulation [17].

Moreover, DEHP has potential to interfere with fetuses by crossing the placenta barrier, leading to developmental anomalies [18] like deceased litter weight [19], short anogenital distance [20-22], hypospadias and cryptorchidism [23, 24], and testicular dysgenesis in males [25]. Toxicological study carried out on the reproductive response of rabbit bucks [15] that established negative impact of DEHP on reproductive parameters has necessitated investigation of its impact on the female rabbit reproductive system.

With the widespread of DEHP in agricultural domains, information about the specific levels at which it becomes deleterious to the reproductive potential and development of female rabbits is still sparse. Majority of the research conducted were based on injections of DEHP at high doses which have failed to demonstrate dietary trace doses common on the farm. There is therefore the quick need to look into the pattern of effects impacted by DEHP on the growth and health of the does, ovarian function and finally, the wellbeing of the kittens.

Consequently, this aim of this study was to assess the influence of varied levels of dietary DEHP on the performance, blood profile, ovarian health (post-mortem) as well as metrics of the kittens.

2. Materials and Methods

2.1. Experimental Site and Ethical Clearance

This experiment was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Ilesa, Ilesa, Nigeria. All the procedures used were with the approval of the University's Animal Care and Use Committee, with conformity to international standards on the handling of laboratory animals.

2.2. Rabbits, Experimental Design and Husbandry

A total of forty-five pre-pubertal rabbit does (crosses) were randomly distributed to five experimental treatments, with nine replicates (1 rabbit each) to ensure equal average body weight across groups. Before grouping, the female rabbits were placed on a control diet, with water available *ad libitum* during a 2-week acclimatization period. The health status of the rabbits was monitored daily throughout the trial. After a 75-day experimental period, 3 does were picked at random from each group for blood sampling and analysis.

2.3. Breeding and Management of Does During Gestation

At day 76, all the rabbits were mated by untreated males. The matings were successful for some and kindling took place at 30±2 days.

2.4. Collection of Data

Initial and final weights, feed intake and body weight gain were recorded. The feed conversion ratio (FCR) was estimated as the ratio of feed intake to weight gain. Blood samples were collected, while hematological and biochemical analyses were conducted following the methodologies outlined by [15] and [26]. After kindling, crown-rump length (CRL) of the kittens was measured from the top of the head (crown) to the buttocks using a thread, the length of which was later taken from a ruler [27], while anogenital distance was measured as the distance from the center of the anus to the genitalia [28] using a ruler.

Ovary weight and number of follicles were determined using the method outlined by [29]. After carefully opening up the abdominal section of the rabbits, left and right ovaries were carefully extracted and weighed individually after which a magnifying hand lens was used to view the ovaries and the follicles were counted. The Graafian follicles were seen raised all through the sides of the ovaries, while the corpus albicans were not raised and dark in colour within the ovaries.

2.5. Hormonal Assay

The test for hormonal parameters in the blood serum was conducted with the aid of the tube-based enzyme immunoassay (EIA) method. The procedure used for the hormonal assay was according to the method of [30] as described for the Kit (BioCheck ELISA Assay, USA)

2.6. Statistical Analysis

Data were analyzed using the ANOVA procedure from the Statistical Analysis Systems Institute (SAS, 1999). Differences between means were determined using Duncan's Multiple Range Test from the same software, at 5% level of significance.

2.7. Formulation of Experimental Diets

DEHP (purity >99.5% Sigma-Aldrich, USA) was introduced into the diets at the following levels of inclusion: T1 - 0 ppm, T2 - 100 ppm, T3 - 200 ppm, T4 - 300 ppm, and T5 - 400 ppm.

Table 1. Gross composition of experimental diet (g/100g).

Ingredient	Composition
Maize	20
Soybean meal	20
Fish meal	1
Palm kernel meal	10.5
Rice Husk	20
Wheat offal	25
DCP*	2
Limestone	1
Vit. mineral premix	0.25
Salt	0.25
Total	100
Calculated analysis	
Crude protein (%)	17.99
D.E (kcal/kg)	2,789.10
Crude fiber (%)	11.33

* Dicalcium Phosphate.

3. Results

3.1. Effect of Dietary DEHP on Performance Characteristics of Rabbit Does

In this investigation, the feed intake of rabbit does under different treatments was evaluated to compute the average daily feed intake, which was found to be: 33.13, 33.03, 33.03, 33.64, and 32.61 g/day for treatments 1, 2, 3, 4, and 5 respectively (Table 2). Results of daily feed intake of the does showed a linear decline across the treatment diets (Table 2). The does that received the control diet had similar daily weight gain rates as those that were given diets with DEHP ($p>0.05$), although there was a steady reduction from 12.45 g/d in the control group to 11.15 g/d in T5, suggesting that the increases in DEHP levels in the diets did not impact the weight of the does. The FCR on the control diet was the lowest at 2.65, while the highest value of 2.96 was observed in rabbit does from treatment 5.

Table 2. Performance characteristics of rabbit does exposed to dietary DEHP.

	Treatments					
	T1	T2	T3	T4	T5	
Parameters	Ctr.	100 ppm	200 ppm	300 ppm	400 ppm	SEM
Initial Live Wt. (g)	964.70	964.00	955.30	955.00	965.70	26.06
Final Live Wt. (g)	1902.00a	1862.70b	1855.00b	1815.70c	1805.00c	25.05
Feed Intake (g/day)	33.13	33.03	33.03	33.64	32.61	0.13
Body Wt. Gain(g/day)	12.45	12.10	11.76	11.65	11.15	0.04
FCR	2.65	2.75	2.75	2.84	2.96	0.07

3.2. Effect of Dietary DEHP on Haematological Indices of Rabbit Does

Presented in Table 3 below are the haematological parameters of rabbit does that consumed DEHP contaminated diets. The statistical analysis revealed that all haematological parameters [packed cell volume (PCV), haemoglobin, red blood cells (RBC), white blood cells (WBC), platelets, Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), and Mean Cell Volume (MCV)] remained unaffected by the treatment.

Table 3. Haematological indices of rabbit does exposed to dietary DEHP.

	Treatments					
Parameters	T1	T2	T3	T4	T5	SEM
	Ctr.	100 ppm	200 ppm	300 ppm	400 ppm	
PCV (%)	40.00	39.00	36.50	35.00	35.00	0.23
Haemoglobin (g/dl)	13.20	12.60	12.25	11.80	11.30	0.07
RBC (x103/µl)	13.33	13.02	12.98	12.78	12.69	0.08
WBC (x106/µl)	6.50	6.45	6.40	6.20	5.95	0.03
Platelets (x106/µl)	121.00	120.50	113.50	111.00	105.00	0.60
MCHC (%)	33.81	33.55	33.00	32.60	32.34	0.28
MCH (pg)	90.02	96.88	94.37	92.55	90.63	0.39
MCV (µ3)	30.01	29.99	28.12	28.02	27.69	0.29
Lymphocytes (%)	64.00	69.00	63.00	63.50	67.50	0.26
Neutrophil (%)	32.00	26.50	33.50	32.50	29.50	0.28
Monocytes (%)	1.50	1.50	2.00	2.00	2.50	0.08
Eosinophil (%)	2.50	3.00	1.50	2.00	3.00	0.08

Means with no superscripts are statistically simiar ($p>0.05$).
PCV=Packed Cell Volume; RBC=Red Blood Cell; WBC=White Blood Cell; MCHC= Mean Corpuscular Hemoglobin Concentration; MCH = Mean Corpuscular Hemoglobin; MCV = Mean Corpuscular Volume; SEM= Standard Error of Means

3.3. Effect of Dietary DEHP on Serum Biochemical Parameters of Rabbit Does

The influence of diets that contained DEHP on the serum biochemical values of does is detailed in Table 4. The dietary inclusion of DEHP did not affect ($p>0.05$) any of the parameters {Alkaline Phosphatase (ALP), Cholesterol (CHOL), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL)} except for Aspartate amino transferase (AST) and Alanine amino transferase (ALT) which were significantly increased ($p<0.05$). The AST value for does on the control diet was lower than that of those on DEHP-containing diets. In T2, the AST value was recorded as 92.19 I.U/L, in the T3 group it was 93.97 I.U/L, in the T4 group it was 93.97 I.U/L, and in the T5 group it was 99.47 I.U/L. Furthermore, it was noted that the AST values increased as the level of DEHP inclusion rose in all treatments.

Table 4. *Serum biochemical parameters of rabbit does exposed to dietary DEHP.*

	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	
Parameters	**Ctr.**	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**SEM**
Total Protein (g/dl)	7.27	8.37	7.69	6.11	9.80	0.20
Albumin (g/dl)	3.30	3.27	3.22	3.04	2.96	0.02
Globulin (g/dl)	3.97	5.10	4.47	3.07	6.84	0.22
Creatinine (mg/dl)	1.35	1.40	1.55	1.70	1.35	0.01
Urea (mg/dl)	18.56	19.02	19.47	20.05	20.87	0.12
AST (I.U/L)	33.65a	92.19b	93.97b	93.97b	99.47b	0.74
ALT (I.U/L)	16.17a	17.70a	22.67b	22.92b	31.03c	0.38
ALP (I.U/L)	99.86	98.60	93.70	91.92	81.21	1.26
CHOL (mg/dl)	60.12	70.20	72.05	79.80	87.28	1.04
HDL (mg/dl)	35.58	29.83	24.42	35.70	27.68	0.44
LDL (mg/dl)	9.27	9.56	8.87	10.89	8.42	0.11

[a,b,c] means on the same row with different superscripts are statistically different ($p<0.05$). SEM = Standard Error of Means; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein;

3.4. Effect of Dietary DEHP on Hormone Production of Rabbit Does

The hormone production in rabbit does that were administered different levels of DEHP is illustrated in Table 5. There were no significant ($p>0.05$) changes in the concentrations of follicle stimulating hormone (FSH) and progesterone in the female rabbits. Nonetheless, differences noticed in the levels of Luteinizing Hormone (LH) and estradiols were significant ($p<0.05$). The LH levels in rabbit does from T1 and T2 were similar ($p>0.05$) and likewise, LH levels in T3 and T4 also did not show significant differences ($p > 0.05$). The least LH value was found in T5, with a mean value of 10.50 I.U/L. Furthermore, the mean estradiol level in T1 was significantly ($p<0.05$) higher than those in the other groups, while T5 had the lowest value. The concentration of estradiol was lower at higher inclusion levels of DEHP.

Table 5. *Hormone production of rabbit does exposed to dietary DEHP.*

Treatments						
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
	Ctr.	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	
LH	15.00a	15.00a	12.50ab	12.50ab	10.50b	0.09
FSH	12.00	11.00	9.50	8.50	8.50	0.16
Progesterone	5.05	4.90	4.90	4.85	4.75	0.02
Estradiol	24.35a	21.95b	20.80bc	20.15bc	19.85c	0.05

[a,b,c] means on the same row with different superscripts are statistically different ($p<0.05$). SEM = Standard Error of Means; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone

3.5. Effect of Dietary DEHP on Metric Parameters of Rabbit Kittens Exposed to Varied Levels of DEHP *in utero*

Table 6 presents the metric parameters of the rabbit kitten after *in utero* exposure to DEHP. The litter size, anogenital distance and crown- rump length were significantly ($p<0.05$) highest in the control group. Reproductive failures were also observed at T4 and T5.

Table 6. *Metric Parameters of Rabbit Kittens Exposed to varied Levels of DEHP in utero.*

	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	
Parameters	**Ctr.**	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**SEM**
Litter Size	5.00a	3.00b	3.00b	0.00c	0.00c	0.00
Litter Weight (g)	68.30a	70.76a	68.71a	0.00b	0.00b	0.07
AGD (cm)	0.75a	0.35b	0.30b	0.00c	0.00c	0.00
CRL (cm)	11.25a	8.05b	7.65b	0.00c	0.00c	0.04

a,b,c, means on the same row with different superscripts are statistically different ($p<0.05$). SEM = Standard Error of Means. AGD = Anogenital Distance, CRL = Crown -Rump Length, ppm = part per million (equivalent of mg/kg)

3.6. Effect of Dietary DEHP on Ovary Weight and Number of Follicles of Rabbit Does

Table 7 describes the ovarian condition after DEHP exposure. A significant ($p<0.05$) decline was observed in the number of Graafian Follicles at the left ovary with the least number at T4 and T5.

Table 7. Ovary Weight and Number of Follicles of Rabbit Does Exposed to varied Levels of Dietary DEHP.

Treatments						
Parameters	T1 Ctr.	T2 100 ppm	T3 200 ppm	T4 300 ppm	T5 400 ppm	SEM
Left Ovary						
Weight (g)	0.14	0.14	0.10	0.08	0.07	0.00
No. of G.F	13.50a	6.00ab	5.00ab	3.50b	3.50b	0.22
No. of T.F	5.00	3.50	2.50	2.00	2.00	0.08
No. of C.A	1.00	0.50	1.00	0.50	0.50	0.06
Right Ovary						
Weight (g)	0.24	0.13	0.08	0.08	0.07	0.00
No. of G.F	8.50	7.00	5.50	4.00	2.50	0.30
No. of T.F	8.00	6.50	6.00	3.50	3.00	0.15
No. of C.A	2.50	1.00	1.00	0.50	0.50	0.16

a,b, means on the same row with different superscripts are statistically different ($p<0.05$). SEM = Standard Error of Means; G.F = Graafian Follicles, T.F = Tertiary Follicles, C.A = Corpora Albican

4. Discussion

The significant reduction observed in the final live weight may suggest that at high level of DEHP dosage, there may be diversion of metabolic energy from tissue accumulation to the pathway of liver detoxification. This is because DEHP possesses the affinity to disrupt the Peroxisome Proliferator-Activated Receptors (PPARs) which coordinate the metabolism of lipid and energy homeostasis [31].

The average daily feed intake of the experimental rabbit does was also reduced, similar to the bucks [15], although the decrease observed was not significant across all treatments. The feed intake of rabbit does at 100, 200, 300, and 400 ppm DEHP was calculated to be 99.76, 99.64, 98.49, and 98.43% of the mean value of the control group. Although the feed intake is stable across all the groups, the feed conversion ratio rose, with T5 having the highest value (Table 2). This rise in the FCR mean values may imply metabolic inefficiency as a result of the ingestion of DEHP. The consequence is that the rabbits ingested the same quantity of feed nutrients but failed to convert the feed nutrients to body mass as a result of oxidative stress induced by MEHP (a metabolite of DEHP) which elevated the rabbits' requirement for maintenance energy [32]. This result is consistent with the findings of [33] and [34], which indicated that DEHP did not have a significant impact on feed intake among the experimental animals. However, the results from this study contrast with those of [35], where a significant increase in feed intake was observed in female mice.

Though not significant, the consistent decline in average daily body weight gain may be due to poor diet utilization resulting from the presence of DEHP in the diets. Studies have implicated DEHP for the disruption of thyroid hormone which primarily controls basal metabolic rate, and this might have resulted in the growth rate decline in spite of the stable feed intake across the treatments [36]. This finding aligns with the studies conducted by [33] and [37], which found that DEHP had no effect on the body weight of cynomolgus monkeys and mice, respectively. However, this result is inconsistent with the findings of Holtcamp [38], which indicated that DEHP had a tendency to increase body weight more than the control group.

The FCR values demonstrated that the feed was inadequately utilized, which can be ascribed to the toxic effects of phthalate. Schoneker *et al*. [39] reported 'irritation, laxation, colitis with erosions and submucosal fibrosis in the gastrointestinal tract in dogs, while ulcers, polyps, and cecal wall thickening were induced in rats'. These factors may account for the observed poor feed utilization as DEHP levels increased in the diets.

Furthermore, even though the erythrogram parameters of the rabbits were not significantly affected, a closer look reveals a drop in the PCV and Hb values of the treatment groups relative to the control group, suggesting a progressive anemia probably induced by MEHP that focuses on the destruction of erythrocyte membrane, thereby leading to premature hemolysis. When this is considered together with the declining values of MCV and Hb, it can be concluded that DEHP disrupts the

heme synthesis. These results are consistent with those of Kwack *et al*. [40] and Ahmadivand *et al.* [41], who found no significant changes in hematological parameters among any treatment groups of rats. Conversely, the findings contrast with those of Da-qiang *et al*. [42], George *et al.* [43], and Olatundun and Ogunlade 15, who asserted that the haematological indices of rabbits and fish were significantly affected by DEHP and Bisphenol A. The discrepancies in these studies may be as a result of differences in species, breed, sex and the nutritional provisions given to the experimental animals.

The significant impact of DEHP on the serum biochemistry of rabbit does was observed in Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) where the treatment groups exhibited significantly different values compared to the control. The increase in the AST value from 33.65 in T1 to 99.47 IU/L in T5 which is about three fold increase, and the rise in ALT value from 16.17 in T1 to 31.03 IU/L in an indication of serious hepato-cellular damage.

This finding corroborates the observations made by Pereira *etal*. [44], Milošević *etal*. [45], and Olatundun and Ogunlade [15]. Kwack *et al*. [40] and Pereira *et al.* [44] also noted increased AST levels in phthalate-treated rats. These heightened levels of liver enzymes AST and ALT indicate potential liver cell damage due to DEHP consumption [46].

This study's findings indicate that DEHP has a detrimental effect on LH and estradiol secretion in rabbits consuming DEHP-contaminated diets, as their levels significantly decreased with higher DEHP inclusion. The declining trend of LH indicates that DEHP could disrupt pre-ovulatory availability and rabbits, being induced ovulators, could experience anovulation. Likewise, a similar trajectory followed by FSH values could be responsible for the reduction in the number of follicles. In the absence of FSH, primordial follicles refuse to transform to antral stages, thereby triggering follicular atresia which characterizes toxicity of DEHP. Moreover, the drastic reduction in the estradiol levels is possibly as a result of the tendency of DEHP to prohibit the availability of aromatase which is an enzyme that plays a role in transforming androgens to estrogens in the granulosa cells. These results are consistent with the research findings of Liu *et al.* [47], which found that the concentration of luteinizing hormone was lower in DEHP-treated rats relative to the control group, while the average values of steroid hormones, including progesterone and estradiol, were significantly diminished in rats on DEHP-contaminated diets. Supporting this, Hannon and Flaws [19] and Parillo *et al.* [48] noted that the ovary is one of the organs impacted by phthalate toxicity, which can lead to a substantial reduction in the production of sex steroid hormones. They reported that steroidogenic enzymes in the corpora lutea are adversely affected, resulting in insufficient production of progesterone and estradiol necessary for maintaining pregnancy. Lovekamp-Swan and Davis [49] discovered that phthalate exposure negatively influenced the female reproductive system, leading to decreased estradiol levels, an extended estrous cycle, and the absence of ovulation in adult rats.

At lower inclusion levels (100 and 200 ppm), DEHP caused impairment of physical growth of the kittens as evidenced in the significant decline in the AGD and CRL. Moreover, the reproductive failure observed at inclusion levels 300 and 400 ppm is an indication of absolute infertility which could have been caused by ovulation failure or fetal resorption. The drop in the AGD of the kittens affirms the anti-androgenic impact of phthalates generally [50]. Phthalates have the potential of disrupting the synthesis of fetal androgen, which can lead to short AGD and result in destruction of reproductive ability of the kitten [50].

The observed reduction in the crown-rump length when viewed alongside similar litter weight values at T2 and T3 might indicate that although the kittens had the same weight as the control, there is a possibility that their skeletal development was compromised. This might be due to the impact of the DEHP on the placenta which could had restricted the flow of needed micronutrients to the fetuses [51].

In addition, there seems to be a correlation between the litter size, follicular atresia and follicle stimulating hormone level earlier discussed. This may be due to the failure of the does to mobilize the required level of follicles which resulted in reduced ovulation rate.

The histomorphometrics presented in Table 7 provides the basis for the reproductive failure discovered in Table 6. The decrease in ovarian weight and total follicular mass, though not significant, suggests that DEHP can cause ovarian dysgenesis in rabbit does [52]. On the right ovary, the sharp reduction in the ovarian weight from 0.24g at T1 to 0.07g at T5, representing about 71% decrease is an indication of serious atrophy and a gradual erosion of the parenchyma. The reason might be because DEHP inhibited gonadotrophins (LH and FSH) synthesis (Table 5) which is responsible for sustaining ovarian tissue size and folliculogenesis [53].

The significant decrease in the Graafian Follicles count at the left ovary signifies follicular depletion. The change from Tertiary Follicles to Graafian Follicles us usually a function of the amount of estradiol and follicle stimulating hormone. However, with the activities of DEHP that limited the functionality of aromatase enzyme, the follicles could not develop into maturity but instead, there was cell death (Hannon *et al*., 2015). This is another explanation for the reduced or no litter size recorded earlier.

5. Conclusion

The results from this study clearly indicate that chronic exposure of rabbit does to Di-(2-ethylhexyl) phthalate (DEHP) via oral administration can produce multidimensional harmful effects on the growth performance, physiological health and reproductive potential of female rabbits. The recorded reduction in the final live weight and the increase in feed conversion ratio are indicative of metabolic inefficiency and redirection of energy to hepatic detoxification pathways. Moreover, the

significant rise in liver enzymes, especially AST and ALT, signifies a serious hepatocellular damage induced by the intake of DEHP.

Furthermore, this study recognizes DEHP as a destructive reproductive toxin in rabbits. It disrupted the Hypothalamic-Pituitary-Gonadal (HPG) axis, thereby inhibiting important gonadotropins and steroid hormones, particularly LH and estradiol, resulting to follicular atresia and ovarian deterioration. This endocrine disruption led to total infertility at exposure levels of 300 ppm and above. Even at lower inclusion levels, the recorded developmental abnormalities in kittens, like reduced anogenital distance and crown-rump length, show significant endocrine disruption *in utero*. These findings stress the quick need for total observance of phthalate contamination in the agricultural value chain to maintain good animal health and ensure sustainable livestock production.

Abbreviations

DEHP	Di-(2-Ethylhexyl)Phthalate
FCR	Feed Conversion Ratio
AST	Aspartate aminotransferase
CRL	Crown Rump Length
DCP	Dicalcium Phosphate
PCV	Packed Cell Volume
RBC	Red Blood Cell
WBC	White Blood Cell
MCHC	Mean Cell Haemoglobin Concentration
MCH	Mean Cell Haemoglobin
MCV	Mean Cell Volume
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
CHOL	Cholesterol
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
AGD	Anogenital Distance
G.F	Graafian Follicles
T.F	Tertiary Follicles
C.A	Corpora Albican+

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Conflicts of Interest

The authors declare no conflicts of interest.

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