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Effect of di-(2-ethylhexyl) phthalate on growth performance, organ weight and carcass characteristics of rabbit bucks

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Abstract

Phthalates are environmental contaminants found in most of consumer products like clothing, pharmaceuticals, building materials and many foods. One of the most commonly used phthalates, di-(2-ethylhexyl) phthalate (DEHP) has been implicated in hepato- and nephro toxicity. In this study, a total number of forty five 10-week old male growing crossbred rabbit buck with average body weight of 1.37 ± 0.24 kg were used to investigate the effect of DEHP on the growth performance, organ weight and carcass characteristics. The rabbits were randomly allotted to 5 dietary treatments. Control diet (T_1) contained no DEHP while T_2, T_3, T_4 and T_5 contained DEHP at 100, 200, 300 and 400 ppm/kg diet respectively. The rabbits were housed individually and fed their respective diets for 75 days. The results showed that initial and final live weight, feed intake, body weight gain and feed conversion ratio were statistically similar ($p > 0.05$) for all the dietary treatments. The DEHP inclusion in the diets of rabbit bucks elicited significant differences ($p < 0.05$) in both the absolute and relative weights of liver, while a significant decrease ($p < 0.05$) in paired kidney weights as inclusion levels increased was observed. In addition, there were significant differences ($p < 0.05$) in absolute weights of spleen as the mean absolute weight values were higher in T_2, T_3, T_4 and T_5 than T_1, while no significant differences were observed in the mean absolute weights of lungs and hearts ($p < 0.05$). Also, significant differences ($p < 0.05$) were recorded in the absolute weights of primal cuts like ribs, loin, shoulder, feet, legs, tail, head and abdominal fat, while their relative mean weights were not statistically different ($p > 0.05$). This study showed that DEHP did not significantly affect all the growth parameters and relative weights of all the primal cuts of the rabbits, while internal organs like liver, kidney and spleen were negatively impacted by the dietary DEHP.

Keywords: Di-(2-ethylhexyl) phthalate; Toxicity; Rabbit bucks; Primal cuts; Relative weight.

RUNNING TITLE: Impact of DI-(2-Ethylhexyl) Phthalate on rabbit bucks

Effet du phtalate de di-(2-éthylhexyle) sur les performances de croissance, le poids des organes et les caractéristiques des carcasses de lapins

Resume

Les phtalates sont des contaminants environnementaux présents dans la plupart des produits de consommation comme les vêtements, les produits pharmaceutiques, les

matériaux de construction et de nombreux aliments. L'un des phtalates les plus couramment utilisés, le phtalate de di-(2-éthylhexyle) (DEHP), a été impliqué dans la toxicité hépato- et néphro. Dans cette étude, un nombre total de quarante-cinq lapins croisés mâles âgés de 10 semaines et d'un poids corporel moyen de 1,37 ± 0,24 kg ont été utilisés pour étudier l'effet du DEHP sur les performances de croissance, le poids des organes et les caractéristiques de la carcasse. Les lapins ont été répartis au hasard entre 5 traitements alimentaires. Le régime témoin (T_1) ne contenait pas de DEHP tandis que T_2, T_3, T_4 et T_5 contenaient du DEHP à raison de 100, 200, 300 et 400 ppm/kg de régime respectivement. Les lapins ont été hébergés individuellement et nourris avec leur régime respectif pendant 75 jours. Les résultats ont montré que le poids vif initial et final, la consommation alimentaire, le gain de poids corporel et le taux de conversion alimentaire étaient statistiquement similaires ($p > 0,05$) pour tous les traitements diététiques. L'inclusion du DEHP dans l'alimentation des lapins a provoqué des différences significatives ($p < 0,05$) dans les poids absolus et relatifs du foie, tandis qu'une diminution significative ($p < 0,05$) du poids des reins appariés à mesure que les niveaux d'inclusion augmentaient a été observée. De plus, il y avait des différences significatives ($p < 0,05$) dans les poids absolus de la rate, les valeurs moyennes du poids absolu étant plus élevées à T_2, T_3, T_4 et T_5 que T_1, alors qu'aucune différence significative n'était observée dans les poids absolus moyens des poumons et cœurs ($p < 0,05$). En outre, des différences significatives ($p < 0,05$) ont été enregistrées dans les poids absolus des coupes primaires comme les côtes, la longe, l'épaule, les pieds, les pattes, la queue, la tête et la graisse abdominale, alors que leurs poids moyens relatifs n'étaient pas statistiquement différents ($p > 0,05$). . Cette étude a montré que le DEHP n'affectait pas de manière significative tous les paramètres de croissance et les poids relatifs de toutes les coupes primaires des lapins, tandis que les organes internes comme le foie, les reins et la rate étaient négativement impactés par le DEHP alimentaire.

Mots-clés: Phtalate de di-(2-éthylhexyle); Toxicité; Des dollars de lapin ; Coupes primaires ; Poids relatif.

Introduction

Phthalates are a family of human-made chemicals that have applications in medical, automotive and consumer product industries (Serrano *et al*., 2014). Exposure to these phthalates is facilitated through their ubiquitous use as a plasticizer or solvent in consumer goods, including personal care products, plastics, cleaning materials, pharmaceuticals, clothing, and building materials. Because phthalates are not covalently bonded to the polyvinyl chloride plastics in which they are primarily used, direct contact is not the only source of exposure as phthalates can readily leak into products or evaporate into the environment. Consequently, exposure can occur through ingestion, inhalation, and even dermal contact with contaminated air (Weschler *et al.,* 2015). Phthalates are also exogenous organic pollutants and livestock are exposed to such substances through contact or food intake during the breeding process (Adeel *et al*., 2017). Among the most important phthalates are dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) (Jarosova *et al*., 2009; Kougias *et al*., 2017). Studies on experimental animals have shown that phthalates interfere with the endocrine system inducing developmental and reproductive toxicity (Zhu *et al.* 2018) and produce respiratory symptoms (Cathey *et al.*, 2020). Melvin (2016) reported that empire gudgeons *Hypseleotris compressa*

exposed to endocrine disrupting chemicals (EDCs) like phthalates caused a significant increase in weight gain. Yuan *et al*. (2017) reported that yellow catfish exposed to 0.5 mg/L DEHP significant improved feed intake compared with the control group. Meng *et al*. (2018) reported that DEHP exposure can disturb lipid metabolism at the enzymatic and mRNA levels in yellow catfish. Baralic *et al*. (2017) reported that mixture of phthalates and bisphenol A (BPA) caused changes in lipid profile and liver-related biochemical parameters of rats, significant increase in liver relative weight in rats exposed to DEHP and a slight increase in lung, kidneys, testicles, brain and thyroid gland relative weight was noted in the group treated with the mixture of DEHP, DBP, and BPA. Wood *et al*. (2014) reported incidence of hepatocellular tumors (adenoma and/or carcinoma) in mice exposed to DEHP. Data from epidemiological studies have suggested DBP and its metabolites are associated with a series of adverse reactions in the kidney, and toxicological studies have also confirmed that DBP has potential nephrotoxicity (Kang *et al.,* 2019).

However, studies on the effects of the DEHP on growth and its toxicity on internal organs of rabbits are limited. Therefore, the aim of this experimental study was to determine the effects of DEHP on growth indices, metric characteristics of organs and carcass of rabbit bucks orally exposed to the chemical for 75 days.

Methodology

The experiment was carried out at the Rabbitry Unit of the Teaching and Research Farm, Ekiti State University, Ado Ekiti, Nigeria. The site is located on Latitude $7^{o}37^{l}15^{ll}$N and Longitude $05^{o}13^{o}28^{o}$E with the temperature range of 21 to 28°C. Analytical standard on purity of over 99.5% Di-(2-ethylhexyl phthalate) (DEHP) was purchased from Sigma-Aldrich, USA through Bristol Scientific Company, Lagos, Nigeria Five diets were formulated including the control (diet 1) with crude protein of 17.99 %, crude fibre of 11.33 % and digestible energy of 2,789 kcal/kg. All diets were iso-nitrogenous and iso-caloric. Diets 2, 3, 4 and 5 contained the same ingredients as the control diet but also contained DEHP at inclusion levels of 100, 200, 300 and 400 ppm respectively.

In the experiment that lasted for 75 days, a total of forty five 10-week old weaned rabbits with an initial average weight of 1.37 ± 0.24 kg were randomly assigned to the five dietary treatments. Each treatment had three replicates of three rabbits each which were housed individually in vermin-proof cages in a completely randomized design. Feed and water were provided *ad libitum* for the rabbit bucks. At the end of the feeding trial, three rabbits were randomly selected from each treatment for analyses.

Data Collection

Feed Intake

After the acclimatization period, each rabbit was offered a weighed amount of feed daily. The rabbits were fed in the morning hours between 7:00 - 8:00 am. The animals had access to fresh and clean drinking water always. Left over feed was collected daily into clearly labeled envelopes and weighed weekly with a digital scale. The feed intake was computed by deducting from the quantity served, the weight of left over feed.

Body Weight Gain

At the beginning of the feeding trial, the weight of each rabbit was taken and weekly thereafter using a sensitive weighing scale. Weight gain was obtained by subtracting the initial weight from the final weight.

Feed Conversion Ratio

The feed conversion ratio describes the amount of feed that produced a unit rise in the weight of the rabbits. It was calculated thus:

$$\text{Feed conversion ration (FCR)} \quad \frac{\text{Feed Intake}}{\text{Weight Gain}}$$

Metric Characteristics of Organs and Carcass Analysis

The rabbits were starved of feed overnight but offered with water and live weights were taken. Fifteen (15) rabbit bucks (three from each treatment) were randomly selected, slaughtered and bled properly before skinning. The eviscerated weight was also recorded and expressed as percentage of the live weight. The carcass was dissected into primal cuts namely ribs, loin, shoulder, feet, legs, tail and head which were weighed and recorded as absolute weight which was also expressed as percentage of the live weight (relative weight). The liver, kidney, spleen, heart and lungs were weighed and expressed as percentages of live weight to obtain the relative weight.

Results and Discussion

Performance Characteristics

The results indicated that no significant differences were observed in all the growth parameters of the rabbits at all DEHP inclusion levels (Table 1).

Table 1: Performance characteristics of rabbit bucks fed varied levels of dietary DEHP

	Dietary Treatments					
	T_1	T_2	T_3	T_4	T_5	
Parameters	**Control**	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**SEM**
Initial Live Weight (g)	1366.70	1353.30	1378.30	1350.00	1388.30	24.10
Final Live Weight (g)	1900.00	1900.00	1880.00	1850.00	1810.00	33.40
Feed Intake (g/day)	39.25	39.24	39.24	39.22	39.00	0.15
Body Weight Gain (g/day)	7.11	7.29	6.69	6.66	5.63	0.30
Feed Conversion Ratio	5.52	5.38	5.86	5.89	6.93	0.18

Means with no superscripts along the same row are statistically similar ($p>0.05$)
SEM = Standard Error of Means; ppm = part per million (equivalent of mg/kg)

The non-significant effect of the varied levels of dietary DEHP on the feed intake of the experimental rabbit bucks in this study is consistent with the work of Pugh *et al.* (2000) who reported that treatment of cynomolgus monkeys with 500 ppm Di-isononyl Phthalate and 500 ppm DEHP for 14 days had no significant effect on feed consumption. The result is also in tandem with the work of Mitchell *et al*. (1985) who observed that though not significantly different, there was a linear decline in feed intake of rats fed DEHP at 50, 200 and 1000 mg/kg/day for 31 days. However, the result on feed intake from this study is contrary to the findings of Holtcamp (2012) who reported a significant increase in feed intake of female mice exposed to diets formulated to deliver one of three levels of DEHP (0.05, 5 or 500 mg/kg body weight).

The apparent decline in the average daily body weight gain of the rabbit bucks might not be unconnected with poor utilisation of the diets occasioned by the presence of DEHP in the diets. Similar result had earlier been observed by Pugh *et al.* (2000) that treatment of cynomolgus monkeys with 500 ppm Di-isononyl Phthalate and 500 ppm DEHP for 14 days had no significant effect on body weight. Marsman (1995) also reported that decreased weight gains were noted in dams exposed to 20,000 ppm DBP during gestation and in dams exposed to 10,000 ppm DBP during lactation. Douglas *et al*. (1986) equally observed a reduced gain in weight compared to controls in mice

placed on DEHP at 1/6 LD50 for up to 12 weeks. On the contrary, Holtcamp (2012) observed that DEHP significantly increased body weight compared with control.

The feed conversion ratio indicated poor utilisation of the feed at high level of DEHP which may be due to toxic effect of the phthalate. The results revealed that rabbit bucks in control, 100, 200, 300 and 400 ppm DEHP will consume 4.31, 4.33, 4.50, 5.88 and 6.93 g of feed respectively to gain a unit of body weight each. Schoneker *et al*. (2003) observed irritation, laxation, colitis with erosions and submucosal fibrosis in the gastrointestinal tract in the dog and ulcers, polyps, and cecal wall thickening in rats. This may be responsible for the observed poor utilisation of feed as DEHP inclusion increased in the diets.

Carcass Composition

The carcass composition of rabbit bucks fed dietary DEHP revealed no significant differences ($p>0.05$) in the live weight, bled weight and eviscerated weight (Table 2). This result agrees with the report of Ghosh and Mandal (2008).

Table 2: Carcass composition of rabbit bucks fed varied levels of dietary DEHP

	Dietary Treatments					
	T_1	T_2	T_3	T_4	T_5	
Parameters (g)	**Control**	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**SEM**
Live Weight	1900.00	1900.00	1880.00	1850.00	1810.00	30.00
Bled Weight	1650.00	1650.00	1650.00	1500.00	1650.00	40.00
Eviscerated Wt.	1100.00	1050.00	1200.00	1300.00	1150.00	30.00
Dressing Percentage	57.89	55.26	63.83	70.27	63.54	0.76

Means with no superscripts along the same row are statistically similar ($p>0.05$)
SEM = Standard Error of Means; ppm = part per million (equivalent of mg/kg)

Carcass and Organ Characteristics

Table 3 shows the carcass and organ characteristics of rabbit bucks treated with DEHP. The results showed that no significant differences ($p>0.05$) were observed in all the primal cuts of the rabbits.

This result is consistent with the findings of Zhuo *et al*. (2017) who reported that carcass characteristics like carcass weight, ham weight, ham rate, average backfat, and loin-eye area were not affected in pigs exposed to different levels of bisphenol A (BPA) which is also a plasticizer

Table 3: Relative values of carcass and organ characteristics of rabbit bucks fed varied levels of dietary DEHP

	Dietary Treatments					
	T_1	T_2	T_3	T_4	T_5	
Parameters (g)	**Control**	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**SEM**
Rib	5.90	6.24	5.88	4.02	6.41	0.17
Loin	13.54	10.52	9.81	14.52	14.45	0.47
Shoulder	13.34	9.78	10.20	12.86	12.94	0.43
Feet	2.09	2.10	2.14	2.23	2.09	0.05
Legs	14.9	11.98	10.53	14.64	13.88	0.54
Tail	0.69	0.41	1.10	0.75	0.79	0.09
Head	9.18	10.04	6.98	10.23	10.79	0.19
Liver	2.32^{ab}	2.32^{ab}	2.47^{a}	2.56^{a}	2.63^{b}	0.04
Kidney	0.25	0.23	0.23	0.23	0.23	0.02
Spleen	0.03	0.03	0.04	0.04	0.04	0.01
Lungs	0.67	0.51	0.65	0.62	0.74	0.03
Heart	0.25	0.22	0.23	0.23	0.23	0.02

a,b, means on the same row with different superscripts are statistically different ($p<0.05$).
SEM = Standard Error of Means; ppm = part per million (equivalent of mg/kg)

The observed significant increase in relative liver weight (Table 3) could be as a result of the alterations brought about by DEHP. The liver might have been enlarged in order to increase its capacity for detoxification of the phthalate. This is in agreement with the report of Sandeep and Chen (2018) that continuous oral exposure of rodents to DEHP can cause hepatomegaly, which is due to hyperplasia and hypertrophy of liver parenchymal cells. Increases in liver weight have been shown to occur with peroxisomal proliferators. This also agrees with the findings of Rihani *et al*. (2015) that liver weight of male rabbits exposed to varied levels of DBP was significantly larger than those in the control group. Mitchell *et al*. (1985) equally reported significant liver enlargement in male rats treated with DEHP across all the treatment groups. However, the result contradicts the findings of Pugh *et al.* (2000) who reported that Di-isononyl phthalate and DEHP failed to elicit an increase in liver weight of cynomolgus monkeys.

The reduction in kidney weight might be due to renal toxicity effect of DEHP. Sandeep and Chen (2018) reported that DEHP is nephrotoxic in mice and may exacerbate this pathological change and lead to chronic progressive nephropathy. David *et al.* (2000) also reported that 2 years of DEHP exposure, kidney weights of male mice were significantly lower than those of the controls. George *et al*. (2017) also reported that the histoarchitecture of kidney of DEHP exposed fish species revealed shrinkage of glomeruli, glomerular distortion, vacuolisation of tubular cells, necrosis and atrophy of renal tubules. In their own findings, Mitchell *et al*. (1985) reported that slight alterations were observed in the kidneys of rats treated with 200 or 1000 mg/kg/day of DEHP, namely, enlargement of the droplets (lysosomes) in the cells of the proximal tubule. Higuchi *et al*. (2003) also recorded a decrease in kidney weight of rabbits exposed to DBP *in utero*. All these results are contrary to the findings of Pugh *et al.* (2000) who observed that treatment of cynomolgus monkeys with 500 mg/kg/day DEHP had no effect on the weight of kidney. However, there were no significant differences ($p>0.05$) in the relative mean values of kidney (Table 3).

Significant splenomegaly (enlargement of the spleen) above the animals in the control group was observed in the rabbit bucks treated with DEHP except those in 100 ppm diet. Tsai *et al*. (2012) stated that increased antigen-presenting activity and an increased T-cell proportion in splenocytes might be responsible for spleen enlargement. However the increase in spleen weight is not significant in relative terms. The non-significant relative weight of spleen is in agreement with the findings of Kwack *et al*. (2010) who reported no significant difference in the relative spleen weight of rats treated with DEHP at 500 mg/kg/day for 2 weeks.

Administration of DEHP in the diets of rabbit bucks did not elicit any significant difference on their weights of lungs and hearts at all inclusion levels although there was apparent increase in the lung weight of rabbit bucks placed on 400 mg/kg/day while bucks on the treatment diets had lower heart weighs than those on the control. Nagao *et al.* (2000) also reported no significant difference in the weight of lungs and hearts in rats. In their own study, Nardelli *et al.* (2017) reported that DEHP treatment (300 mg/kg) decreased heart weights in dams. Posnack *et al.* (2012) observed that isolated cardiomyocytes treated with DEHP *in vitro* have increased levels of reactive oxygen species from fatty acid metabolism, possibly resulting in an increase in the susceptibility of the heart to ischemic injury and ventricular dysfunction.

Conclusion

In conclusion, this study demonstrated that

DEHP did not significantly affect feed intake, body weight gain and feed conversion ratio of the rabbit bucks irrespective of DEHP inclusion level, while some of the internal organs investigated particularly liver, kidney and spleen were significantly affected.

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