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Effect of *Tetrapleura tetraptera* pulp on testicular and epididymal characteristics of growing rabbit bucks under different feeding regimes

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Abstract

Antimicrobial resistance is one of the biggest threats to world health, food security and development. Herbal feed additive is of increased choice in livestock production due to the ban on antibiotics, its cost effectiveness and its nutritional and pharmacological benefits cannot be underestimated. Tetrapleura tetraptera could be of great benefit in animal health management because of its anti-bacterial and nutritional properties. This study is aimed at evaluating the effects of T. tetraptera pulp (TTP) on the testicular and epididymal characteristics of growing rabbit bucks under different feeding regimes. Twentymixed breeds of 6-8 weeks old male rabbits (600-800g) were used for the study. Five experimental treatments were designed such that control (T1) was not treated with TTP (either in feed or water), Treatment 2 (T2) contained 2.5mLTetrapleura tetraptera pulp extract (TTPE)/litre of water, Treatment 3 (T3) contained 5.0mLTTPE/litre of water. Treatments 4 and 5 had Tetrapleura tetraptera pulp meal (TTPM) at 0.25g/kg and 0.50 g/kg diet respectively. Feed and water were supplied ad libitum for eightweeks after which three male bucks were randomly selected per treatment, were sacrificed and their right and left testes and epididymis were harvested for evaluation. Testicular and epididymal lengths were measured with the use of transparent ruler, the width and volume of the testes and epididymis were determined with the use of vernier caliper and measuring cylinder (water displacement method) respectively. Testicular and epididymal weights were determined with the use of electronic balance. Results showed that main effect of mode of administration (feed or water) of TTP had no significant ($p>0.05$) effect on testicular parameters. The main effect of level of inclusion showed significantly higher ($p<0.05$) values for mean testes weight (0.76%), mean testes width (0.60cm) and mean testis volume (0.69) for rabbits on 0.25 level of TTP compared to those on 0.50 TTP. Therefore, TTPcould be used up to 0.25(ml/L or g/kg) in drinking water and feed without any adverse effect on the rabbit male reproductive organs however, 0.50 (ml/L or g/kg) inclusions should be cautiously administered.

Keywords: *Tetrapleura tetraptera,* rabbit bucks, Relative paired testes weight, epididymal weight

Effet de la pâte de tétrapleura tétraptera sur les caractéristiques testiculaires et épididymales de la culture des dains de lapin sous différents régimes d'alimentation

Résumé

La résistance aux antimicrobiens est l'une des principales menaces pour la santé mondiale, la sécurité alimentaire et le développement. L'additif des aliments à base de plantes est d'augmenter le choix de la production de bétail en raison de l'interdiction des antibiotiques, de sa rentabilité et de ses avantages nutritionnels et pharmacologiques ne peut être sous-

estimée. Tetrapleura tetrapatera pourrait être d'un grand avantage dans la gestion de la santé animale en raison de ses propriétés antibactériennes et nutritionnelles. Cette étude vise à évaluer les effets de T. tetrapatera Pulpe (TTP) sur les caractéristiques testiculaires et épididymiques de la culture du lapin de lapin sous différents régimes d'alimentation. Des races vingtages de 6 à 8 semaines de lapins mâles (600-800g) ont été utilisées pour l'étude. Cinq traitements expérimentaux ont été conçus de telle sorte que le contrôle (T1) n'a pas été traité avec TTP (en alimentation ou dans l'eau), le traitement 2 (T2) contenait un extrait de pâte de tétraptera de 2,5 mltetrapleura (ETTP) / litre d'eau, traitement 3 (T3) contenait 5,0 MLTTPE / litre d'eau. Les traitements 4 et 5 avaient des repas de pâte de pulpe tétrapléeura tetraptera (RTTP) à 0,25 g / kg et 0,50 g / kg régime respectivement. L'alimentation et l'eau ont été fournies par ad libitum pour huit semaines après quoi trois mâles mâles ont été sélectionnées au hasard par traitement, ont été sacrifiés et leurs testicules de droite et de gauche et d'épididymis ont été récoltés pour une évaluation. Les longueurs testiculaires et épididymales ont été mesurées avec l'utilisation de la règle transparente, la largeur et le volume des testicules et de l'épididyme ont été déterminées avec l'utilisation de l'étrier vernier et du cylindre de mesure (méthode de déplacement d'eau) respectivement. Les poids testiculaires et épididymiques ont été déterminés avec l'utilisation de l'équilibre électronique. Les résultats ont montré que l'effet principal du mode d'administration (alimentation ou eau) de TTP n'avait aucun effet significatif (p> 0,05) sur les paramètres des testicules. L'effet principal du niveau d'inclusion a montré des valeurs significativement plus élevées (p <0,05) pour le poids moyen des testicules (0,76%), la largeur des testicules moyennes (0,60 cm) et le volume de testicule moyen (0,69) pour les lapins sur 0,25 niveau de TTP comparé à ceux qui sont sur 0,50 TTP. Par conséquent, il devrait être utilisé jusqu'à 0,25 (ml / l ou g / kg) dans de l'eau potable et nourrir sans effet défavorable sur les inclusions de la reproduction masculine de lapin, 0,50 (ml / l ou g / kg).

Mots-clés: Tetrapléeura tetrapatera, daims de lapin, relatif capla testes poids, poids épididymal

Introduction

Phyto-biotics are herbal feed additives of plant origin incorporated in animal diets to improve their production through the enhancement of feed properties, promoting animal production performance and improving the quality of product derived from these animals (Windisch *et al*., 2008). Herbal feed additive is of increased choice in livestock production due to the ban on antibiotics, its cost effectiveness and its nutritional and pharmacological benefits (Muneedra *et al*., 2014). Phyto-biotics generally include herbs, spices as well as essential oils and extracts of plants. *T. tetrapterais a plant of interest* which is generally found in the lowland forest of tropical Africa. The fruit consist of a fleshy pulp with a small, brownish black seeds. It is an indigenous fruit species used extensively in human diet because of its pleasant aroma (Essien *et al*., 1994). The fruit possess a fragrant, characteristic pungent aromatic odour (Aladesanmi 2007). The use of this spice could be of great benefit in animal health management because of its anti-bacterial and nutritional properties (Okwu *et al*., 2003). The plant is called Prekese in the Twi language of Ghana, Uhio (Uhiokrihio) in the Igbo language of Nigeria and Aridan in South western Nigeria. *T. tetraptera* is used in western African folk medicine in the management of wide variety of diseases including diabetes, inflammation, hypertension, convulsion, leprosy, rheumatism, flatulence, jaundice and fever.

The nutritional value of *T. tetraptera* was assessed by Okwu (2003) who reported the proximate and nutritional values as follows: crude protein, (7.44% - 17.5%), crude lipid (4.98% - 20.36%), crude fibre (17% - 20.24%), carbohydrate (43.18% - 49.06%) and food energy (234.42- 379.48g/cal). The fruit is reported to be rich in minerals and the phyto-chemical screening revealed the presence of tannin, phenolic compounds, saponins, alkanoids, steroids and flavonoids which might be responsible for its varied biological and pharmacological properties. Several experiments have been carried out on the effect of phyto-additives on the testicular characteristics of rabbit bucks. Oguike *et al.*(2019) reported on the impact of *Aspilia africana* on semen and testicular characteristics of rabbit bucks and concluded that the supplementation of *Aspilia africana* leaf meal even at low levels adversely affected semen characteristics,testicular histology and testicular morphometric parameters. Oyeyemi and Okediran (2007) reported increased testicular weights as percentage of soymeal in rabbit feed increased. Chibogwu *et al.* (2017) reported significant increase in testicular parameters of buck rabbits with reduced level of methanolic extract *Securidaca longepedunculata r*oot-bark. Ogbuewu *et al.* (2009) reported that up to 15% neem leaf in the ration of matured rabbits could cause mild depressive effect on spermatogenesis, semen quality and seminiferous tubule diameter. Therefore, this study is aimed at evaluating the effects of *T. tetraptera* pulp on the testicular and epididymal characteristics of growing rabbit bucks under different feeding regimes.

Materials and methods

Experimental site

This experiment was carried out at the Rabbitary unit of the Teaching and Research farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. Ogbomoso lies on latitude $8^0$831.7940"N and longitude 4^o14'42.6696" E. Ogbomoso is 334mm^3 above sea level while the average temperature and annual rainfall is 26.1^{0}C and 1070 mm^3 respectively (Climate data, 2019).

Processing of the test ingredient

Matured *T.* te*traptera* fruits were sourced from Ago Owu farm settlement, Ikoyi, Osun State. The fruits were air dried for 10 days after which it was separated into different components i.e. the pulp, woody shell and the seeds. The pulp was milled to be used as the test ingredient. A portion of the pulp was milled into *T. tetraptera* pulp meal (TTPM) and was incorporated as part of the rabbit's diet.10g of the pulp was soaked in 1 litre of hot water (100^0C) and allowed to stand for six hours before filtration. The *T. tetraptera* pulp extract (TTPE) was administered through drinking water for the rabbits.

Experimental animals and management

A total of twenty mixed breed rabbit bucks (6-8weeks old) weighing between 600-800g were used for the study, which lasted eight weeks. The bucks were randomly allotted into five treatments of four bucks per treatment, (four replicates /treatment). The animals were acclimatized for twoweeks during which they were given prophylactic treatment. The bucks were weighed at the beginning of the study to determine their initial weight and they were weighed on weekly basis to nearest kilogram. Feed offered were weighed on a weekly basis to determine the weekly feed intake per rabbit. The five experimental treatments are as follows: control (0mL, kgTTP), T2 (2.5mLTTPE/L), T3 (5.0mLTTPE/L), T4(0.25gTTPM/Kg) and T5 (5.0gTTPM/Kg).

Table 1: Gross composition of experimental diets (%)

Ingredients	0mL,kg TTP	0.25mL TTPE/L	0.50mL TTPE/L	0.25g TTPM/Kg	0.50g TTPM/Kg
Maize	15.00	15.00	15.00	15.00	15.00
Soyabean Meal	10.00	10.00	10.00	10.00	10.00
Fish Meal	1.50	1.50	1.50	1.50	1.50
Premix	0.25	0.25	0.25	0.25	0.25
Rice bran	30.20	30.20	30.20	30.20	30.20
Palm Kernel Cake	20.00	20.00	20.00	20.00	20.00
Wheat Offal	19.50	19.50	19.50	19.50	19.50
Oyster Shell	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Bone Meal	2.0	2.0	2.0	2.0	2.0
TTPM	-	-	-	0.25	0.50
Total	**100**	**100**	**100**	**100**	**100**
Proximate composition (%)					
Dry Matter	92.09	92.09	92.09	91.80	91.95
Crude Protein	16.40	16.40	16.40	16.56	16.48
Ether Extract	3.90	3.90	3.90	3.11	4.10
Crude Fibre	11.50	11.50	11.50	11.18	11.32
Ash	6.50	6.50	6.50	6.58	6.64
Nitrogen free extracts	53.79	53.79	53.79	53.77	53.41

Premix; growers premix was obtained from AdomFeedmill in Ogbomosho; it contained Vitamin A I.U 10,000 000.00,Vitamin D3 I.U 2,000 000.00, Vitamin E mg 20,000.00,Vitamin K3 mg 2,000.00, Vitamin B1 mg 3,000.00, Vitamin B2 mg 5,000.00, Niacin mg 45,000.00, C alcium pantothenate mg 10,000.00, Vitamin B6 mg 4,000.00, Vitamin B12 mg 20.00, Chloline chloride mg 300,000.00, Folic acid mg 1,000.00, Biotin mg 50.00, Manganese mg 300,000.00, Iron mg 120,000, Zinc mg 80,000.00, Copper mg 8,500.00 Iodine mg 1,500.00, Cobalt mg 300.00, Selenium mg 120.00, antioxidant mg 120,000.00
TTPM-*Tetrapleura tetraptera* pulp meal and TTPE-*Tetrapleura tetraptera* pulp extract

Data collection

After 8weeks of feeding trial, three (3) bucks per treatment were sacrificed, after which the right and left testes and epididymis were harvested from each buck. The following parameters were evaluated: testicular and epididymal weights using a digital sensitive scale, testicular and epididymal lengths with the aid of a transparent ruler, testicular width using a vernier caliper and testicular volume (Archimedes principle). Testes weights were expressed as relative paired testes weights. Right and left testes density was determine using the equation below:

$$\text{Density} = \frac{\text{Paired testes weigh}}{\text{Paired testes volume}}$$

Statistical analysis

Data collected were subjected to one-way analysis of variance using general linear method of SAS (2003). Means were separated using Duncan multiple Range test of the same package.

Results

Bucks administered *T. tetraptera* pulp through water had higher non-significant ($P > 0.05$) values for mean testes length, mean testes width, mean testes volume and mean epididymal length while those bucks with TTPM had higher non-significant ($P > 0.05$) values for paired testes weight, paired epididymal weight and paired testes density as shown in Table 2.

Table 2: Main effect of TTP administration mode on testicular measurements

Parameters	Feed	Water	SEM	P value
Paired testes weight (g)	1.28	1.23	0.05	0.97
Mean testes width(cm)	0.18	0.47	0.03	0.22
Mean testes length(cm)	1.83	1.93	0.06	0.46
Mean testes volume (ml)	0.38	0.63	0.04	0.67
Paired testes density (g/ml)	0.35	0.32	0.10	0.04
Paired epididymal weight (g)	0.37	0.31	0.06	0.54
Mean epididymal length (cm)	0.99	1.12	0.05	0.82

Table 3: Main effect of TTP inclusion levels on testicular parameters

Parameters	0TTP	0.25TTP	0.50TTP	SEM	P value
Paired testes weight (g)	1.20^{b}	1.52^{a}	1.25^{b}	0.06	0.00
Mean testes length(cm)	1.91	1.86	1.88	0.02	0.36
Mean testes width(cm)	0.53^{a}	0.60^{a}	0.33^{b}	0.03	0.00
Mean testes volume (ml)	0.73^{a}	0.29^{c}	0.50^{b}	0.05	0.00
Paired testes density (g/ml)	0.31^{b}	0.41^{b}	1.25^{a}	0.07	0.04
Paired epididymal weight(g)	0.55^{a}	0.43^{b}	0.34^{b}	0.04	0.01
Mean epididymal length (cm)	1.19^{a}	0.95^{b}	1.06^{ab}	0.06	0.04

Table 4: Interaction effect of administration mode and levels on testicular measurements

Interaction	0 TTPM	0.25 TTPM	0.50 TTP M	0 TTPE	0.25 TTPE	0.50 TTPE	SE M	P-value
Paired testes weight(g)	1.20^{c}	1.49^{ab}	1.28^{abc}	1.20^{c}	1.55^{a}	1.23^{bc}	0.04	0.04
Mean testes length (cm)	1.91	1.72	1.83	1.91	1.81	1.93	0.04	0.69
Mean testes width (cm)	0.53^{a}	0.66^{a}	0.18^{b}	0.53^{a}	0.54^{a}	0.47^{a}	0.04	0.00
Mean testes volume (ml)	0.73^{a}	0.38^{b}	0.38^{b}	0.73^{a}	0.20^{b}	0.63^{a}	0.05	0.00
Testes density (g/ml)	0.31	0.43	0.35	0.31	0.42	0.32	0.04	0.15
Paired epididymal weight	0.55	0.48	0.37	0.55	0.38	0.31	0.04	0.48
Mean epididymal length(cm)	1.19	0.99	0.99	1.19	0.91	1.12	0.04	0.14

Table 3 revealed significant ($p<0.05$) higher values in favor of paired testes weight (1.52g) and testes width (0.6 cm) at 0.25 (mL/L and g/kg) TTP levels of inclusion. Bucks on control treatment (0mL/L, 0g/kg TTP) had significantly ($p<0.05$) higher values for mean testes volume (0.73mL), paired epididymal weight (0.55g) and mean epididymal length (1.19cm) while paired testes density was significantly affected ($p<0.05$) at 0.5 (ml/L and g/kg) TTP level of inclusion.

The interaction effect of mode of administration and levels of inclusion (Table 4) showed that TTPE had significant ($P<0.05$) effects on relative paired testes weight, mean testes width and mean testes volume. There were no significant variations based on interaction effects on mean testes length, paired epididymal weight and mean epididymal length.

Discussion

The non-significant differences ($p>0.05$) observed in the inclusion level of TTP (administered via water or feed) on buck testicular parameters, agrees with the result of Akah and Nwambie (1994) who reported

that *S. longependunculata* inclusion improved spermatogenesis at low concentration. Testis size is a good indicator of spermatozoa production in male animals (Morris, *et al*., 1978). The heaviest relative testis weights were observed in buck rabbits placed on0.25TTP (administered via water or feed), thisimplies that the reproductive potential of bucks placed on 0.25(administered via water or feed) TTP will be higher compared to those in control as Oyeyemi, *et al.,* (2002) reported that larger testes (without any abnormality) produced more spermatozoon than smaller testes. Chibuogwu *et al.* (2017) reported 50mg/kg body weight of *S. longepedunculata* root-bark methanolic extract for effective rabbit buck testicular functionality. The author also advocated caution in relation to dosage and length of administration of phyto-chemicals to rabbit bucks. Results from this study agreed with the observation of Chibuogwu *et al.* (2017), as 0.25g/kg TTPM showed better result for paired testes weight and testes width. Oguike *et al.* (2019) evaluated the impact of *Aspilia africana* on semen and testicular characteristics of rabbit bucks at 0, 10 and 20 g/kg feed, it was discovered that the testicular morphometry of the rabbit bucks was adversely affected as supplementation increased. Scrotal circumference, paired testes weight and paired testes volume significantly decreased with increase inclusion levels of A. *africana*, this points to impaired spermatogenesis and reproductive processes. Ewuola *et al.* (2019) suggested feeding rabbits with *Moringa oleifera* leaf meal up to 2.5% as a supplement, improved sperm production and sperm reserves while above this level, it reduced sperm storage potentials and daily sperm production,relativepaired testes weights of 0.27-0.28% was recorded which was lower than the range of 0.60-0.76% in this current study. The variations might be due to the age of rabbit bucks, period of feeding, breed and the test ingredient. Generally, the positive effect of 0.25g/kg TTP on the testis morphometry may have been attributed to its antimicrobial, antioxidant or aphrodisiac properties. The lower values obtained from 0.50 g/kg TTPM for testicular parameters may be due to toxicity at 0.50 g/kg level of inclusion as Mongalo and Mafoko (2013) commented that higher levels of toxic concentration of cassia in diets reduces testicular weight. The main effects of mode of administration of TTP (TTPE or TTPM) on testicular measurements of buck rabbits showed that there were no differences ($p>0.05$) across the treatments. However, numerically higher relative paired testes weight was observed in rabbits placed on TTPM, whereas the lower paired testes weight was observed in rabbits placed on TTPE.Testis length was numerically higher in rabbits fed TTPM, however, lower in rabbits administered TTPE. The result of the interaction between mode of administration and level of inclusion of TTP was shown on Table 4. The result showed that interaction between mode of administration and level of inclusion of TTP has significant effect ($p<0.05$) on paired testes weight, means testes width, mean testes volume and mean testes density. 0.25mL/L TTPE in water and 0.25mg/Kg TTPM in feedhad similar and the highest values among the treatments. The obtained values for paired testes weight (1.52 and 1.25g) of TTPE and TTPM at 0.25 levels in either mode of administration agreed with the claim of Chibuogwu *et al.* (2017) that caution is needed in relation to dosage and length of administration of phyto-chemicals for the improvement of spermatogenesis. Mean testes length obtained from the result of the interaction showed significant ($p<0.05$) effect. All the obtained results were similar except the values obtained from 0.5mg/Kg TTPM. Mean testes volume followed the same trend save 0.25mg/KgTTPM and 0.25ml/L TTPE that

had lesser values (0.38 and 0.20 respectively). Paired testes density also showed no significant difference across the treatments.

Conclusion and recommendation

According to the findings from this study, *Tetrapleura tetraptera* pulp had no adverse effect on the testicular and epididymal characteristics of rabbit bucks when administered through feed or water at 0.25(mL/L or g/kg) and 0.5(mL/L or g/kg) levels. Inclusion at 0.50(mL/L or g/kg) levels compared to those on 0.25 (mL/L or g/kg) appeared to reduce relative paired testes weight and testes width. It could be recommended that *T.tetraptera* pulp should be used up to 0.25(ml/L or g/kg) in drinking water and feed without any adverse effect on the rabbit male reproductive organs however,0.50 (mL/L or g/kg) inclusions should be cautiously administered. Further studies could explore long-term feeding effects and semen production characteristics.

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