INFLUENCE OF ENZYME SUPPLEMENTATION ON RABBITS FED RUMEN LIQUOR WITH POULTRY WASTE FERMENTED CASSAVA PEELS BASED DIETS

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ABSTRACT

The effect of increasing the optimum replacement level of maize with rumen liquor fermented cassava peels (RLFCP) with enzyme (E) supplementation in rabbit diets was evaluated in 56-day feeding trial using a 2 x 3 factorial experimental design. The E supplementation significantly increased (p<0.05) final live weight (FLW) and total weight gain (TWG), while the RLFCP inclusion (0 – 100 %) led to significant decrease (p>0.05) in the rabbit FLW, TWG and feed conversion ratio (FCR). However, RLFCP inclusion at various levels significantly increased (p<0.05) the daily feed intake (DFI). The slaughtered weight, skin, head and limb, kidneys, gall bladder and gastro intestinal tract were significantly affected (p<0.05) by dietary RLFCP levels. RLFCP inclusion significantly increased (p<0.05) packed cell volume (PCV), mean cell volume (MCV) and lymphocyte (LYM) levels. Enzyme x RLFCP was significant increased (p<0.05) the number of red blood cells. Equally, the concentrations of albumin, globulin, alkaline phosphatase, amylase and glucose were significantly affected (p<0.001) by the level of RLFCP in the diets such that replacement level of maize for RLFCP lead to increase in albumin and glucose, alkaline phosphate, while the effect of the replacement levels did not follow a particular trend for amylase and glucose. Addition of multi-enzyme at 0.35 g/kg level led to improvement in weight gain of the rabbits.

Keywords: Fermented cassava peels, Rabbits, Enzyme supplementation, Blood profiles, Health status

INTRODUCTION

Rabbit production has been identified as one of the ways of solving the ever-increasing problem of animal protein shortage in developing countries among which are Nigeria and sub-Sahara African countries (Ajala and Balogun, 2004). This is because rabbits possess numerous good potentials and attributes such as high growth rate, high prolificacy, high protein forage conversion to meat efficiency and relatively low production cost. However, the high cost of feed has been a major limiting factor to the intensive rabbit production. The feed production cost for rabbit production under intensive production system is very high due to high cost of feed ingredients (Adeyemi *et al*., 2008). For instance, maize, which is the main energy source in rabbit ration is now very expensive and scarce because of the seasonality of its production, high demand for it by man as food and its use in brewing and other industries and recently as major ingredient in bio-fuel/diesel production (Esonu *et al.,* 1999; Folayan, 2013). Maize starch are hydrolyzed and enzymatically treated or fermented to produce syrups, particularly high fructose corn syrup, sweeteners and alcohol (Folayan, 2013). The rise in price of maize in Nigeria indicates a higher demand and low supply due to low grain

yield compared with the world average (Faniyi and Ologhobo, 1999; Folayan, 2013). The search for alternative cheap dietary ingredients, which are not consumed by man to feed livestock and reduce the strife competition between man and animals for some food, becomes very necessary (Sucharita *et al.,* 1998). The use of by-products of agro-industrial origin as low cost alternative carbohydrate sources for livestock nutrition has become more popular (Delgado-Vargas and Parades-Lopez, 1997).

Cassava waste is one of the cheapest and most available potential replacements for maize as it is found all year round in cassava processing centre left as wastes (Ifut, 1998). Unlike most other non-conventional feed resources, cassava is available all year round (FAO, 1994).

Nigeria produces 37,504,100 tonnes of cassava which is the highest in the world (FAO, 2013). For instance, in 2005 Nigeria produced 34 – 40 million metric tonnes of cassava tubers (Aro *et al*., 2010), with peels constituting 8 % of the whole cassava root (Tewe, 1996). This translated to the generation of 2.96 million metric tonnes of the peel per annum, most of which were discarded as wastes and left to rot away. Cassava peel contains 179 g/kg dry matter, 420 g/kg crude protein and 326 g/kg fat. It is relatively high in crude fibre (296 g/kg), ash (747 g/kg) and 555 g/kg nitrogen free extract (Aro *et al.,* 2010).

The toxic level of cynogenic glycosides, the low protein level and high fibre level affect utilization of cassava wastes (Iyayi and Tewe, 1994). Previous reports on rabbit production indicated that cassava peels exerts some negative effects on performance and health status (Oluremi and Nwosu, 2002; Oloruntola *et al*., 2016a). Therefore, various processing methods such as sun drying, retting, fermentation and enzyme supplementation among others are being considered to enhance the nutritional value and utilization of cassava wastes. Earlier reports showed that cassava peels meal nutritional value could be enhanced by fermentation with rumen liquor (Oloruntola *et al.,* 2015) and that rumen liquor fermented cassava peels (RLFCP) could be used to replace maize up to 50 % in weaner rabbit's diets (Oloruntola *et al*., 2016a). Some positive results have been reported when rabbits' diets are supplemented with enzymes (Fernandez *et al*., 1996; Bolis *et al*., 1996; Eiben *et al*., 2004; Ayodele *et al*., 2016). In a preliminary experiment (Oloruntola *et al*., 2016a), maize was replaced with RLFCP at varying levels. The best rabbit's performance was at 50 % maize replacement level with RLFCP. This prompted further study into the effect of enzyme supplementation on rabbits fed diets whose maize in being replaced with RLFCP at higher levels beyond 50 %. It is therefore conceived that optimum replacement value of RLFCP in rabbit's diet can be increased beyond 50 % by multi-enzyme supplementation. The utilization of fermented cassava peel for animal feeding will enhanced growth performance and proffer solution to problem of animal protein shortage being experience in the developing countries, and particularly in regions such as Africa, Asia and South America where cassava waste is available and underutilized (IFAD/FAO, 2000). Therefore, this study aimed at studying the influence of multi-enzyme supplementation on rabbits fed diets whose maize is being replaced with high levels of RLFCP.

MATERIALS AND METHODS

This 56-day feeding trial was carried out at the Teaching and Research Farm of the Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria.

Experimental Design and Animal Management: One hundred and eighty healthy, five weeks old weaner rabbits of crossbreeds and mixed sexes were randomly allotted to the six dietary treatments after balancing for weight in a completely randomized design. Each treatment group was replicated ten times with three weaner rabbits representing a replicate. The rabbits were housed individually in three-tiers, wooden framed and wire meshed cages. The cages were raised 90 cm above the ground and housed in well-ventilated pen. Each rabbit was provided with separate galvanized water trough and feeder.

Experimental Diets: Exogenous enzyme (E) used in this study was manufactured by Bioproton PTY Limited, Brisbane Qld. Australia. The composition of the commercial endogenous enzyme is as shown in Table 1. Fresh cassava peels were collected from the cassava processing cottage industries in Akure, Nigeria and processed as earlier described by Oloruntola *et al.* (2015; 2017). Thereafter, the peels were washed with water, sun-dried, ground and stored. Droppings of commercial layers fed layer's mash were collected from laying unit of the Teaching and Research Farm of the Agricultural Technology Department of the Federal Polytechnic, Ado Ekiti, sun-dried, milled and kept in a cool dried place until used. The rumen liquor was obtained from twenty freshly slaughtered cattle at the Central Abattoir, Ado Ekiti, Nigeria. The rumen liquor was sieved using muslin cloth to remove undigested food and used immediately. Mixed microbes utilize non protein nitrogen and proteins of low quality substrate for synthesis of microbial protein and improve the protein quality (Oloruntola *et al*., 2015). Therefore, poultry droppings (nitrogen sources) was mixed with cassava peels meal to provide the nutritional requirements of micro-organisms being introduced in a solid state fermentation process in this study (Noonhorn *et al*., 1992). Ground cassava peels were mixed with autoclaved layer dropping at the rate of 100 gkg^{-1} of cassava peels meal in black polythene bag, sprayed with freshly collected bovine rumen liquor at rate of 250 ml/kg and fermented anaerobically for 7 days. The fermented cassava peels were sundried for four days and analysed for proximate composition (AOAC, 1995). Six experimental iso-caloric and iso-nitrogenous diets (Table 2) were formulated and designated as diet 1 (Positive control; 0 % RLFCP-E), diet 2 (negative control; 0% RLFCP+E), diet 3 (75 % RLFCP-E), diet 4 (75 % RLFCP+E), diet 5 (100 % RLFCP-E) and diet 6 (100 % RLFCP+E). Diets 1, 3 and 5 were not supplemented with endogenous enzyme but had their maize replaced by RLFCP at 0, 75 and 100 % respectively; while diets 2, 4 and 6 were supplemented with endogenous enzyme and had their maize replaced by RLFCP 0, 75 and 100 % respectively. The diets were pelletized (4 mm diameter and 8 mm long). The experiment was carried out in a 2 x 3 factorial experiment i.e. 0 and 0.35 g/kg E levels x 0, 75 and 100 % RLFCP inclusion levels.

Performance Criteria: The weekly weight gain, feed consumption, feed conversion ratio, haematological and serum biochemical indices were obtained as described by Oloruntola *et al.* (2016a). The weight gains of the rabbits were obtained by subtracting the initial weight from the final weight. The feed consumption was calculated as the difference between daily feed given and left over. The feed conversion ratio was the ratio of feed consumed to the total weight gain of the rabbit. At the end of 8 weeks of the feeding, ten randomly selected rabbits from each dietary treatment groups were tagged, starved overnight, slaughtered and skinned following the guidelines of World Rabbit Science Association (Blasco *et al.,* 1993). The skin, head and limbs were removed, weighed and expressed as percentage of slaughtered weight. Furthermore, the lung, liver, kidney, heart, pancreases, gall bladder and gastrointestinal tract were removed, weighed separately and expressed as percentage of slaughter weight. Dressed weights were determined and used to calculate the dressed percentage for the rabbits. The hind legs, forelegs, breast and rib cage, loin and abdominal wall and neck were also removed, weighed and expressed as percentage slaughter weight.

Blood samples were collected from the prominent ear vein of the rabbits into two separate bottles; potassium ethylene diamine tetra acetic acid (K-EDTA) bottle and a plain bottle for haematological studies and serum metabolite analysis, respectively. The haematological parameters; packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), haemoglobin concentration (HBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets (PLA), lymphocytes (LYM), monocytes (MON) and granulocytes (GRA) were determined using Shenzhen Mind Ray Auto Haematology Analyzer, Model Bc-3200 (Shenzhen Mind Ray Biomedical Electronics Company, Hamburg 20537, Germany).

Table 1: Composition of commercial exogenous enzyme used for supplementing rumen liquor with poultry waste fermented cassava peels based diets fed to rabbits

Composition	Amount/g
Alpha Amylase/*Bacillus subtilis*	400 µ/g
Beta-Glucanase/*Trichoderma longibrachiatum*	700 Bioproton bu/g
Phytase/*Aspergillus niger*	130 µ/g
Cellulase/*Trichoderma longibrachiatum*	6,000 Bioproton µ/g
Zylanase/*Trichoderma longibrachiatum*	10,000 Bioproton X µ/g
Protease/*Aspergillus niger*	700 µ/g

Source: Bioproton PTY Limited. Brisbane Qld. Australia

Table 2: Gross and analyzed composition of the experimental diets

Ingredients (%)	Level of RLFCP Inclusion (%)					
	0-E Diet 1	0+E Diet 2	75-E Diet 3	75+E Diet 4	100-E Diet 5	100+E Diet 6
Maize	43.00	43.00	10.75	10.75	00.00	00.00
RLFCP	00.00	00.00	32.25	32.25	43.00	43.00
Maize husk	22.40	22.40	22.40	22.40	22.40	22.40
Wheat offal	8.00	8.00	8.00	8.00	8.00	8.00
BDG	10.00	10.00	10.00	10.00	10.00	10.00
Soya bean meal	14.85	14.85	14.85	14.85	14.85	14.85
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
Crude protein (%)	15.44	15.44	16.11	16.11	16.33	16.33
Crude fibre (%)	11.33	11.33	12.56	12.56	12.97	12.97
Lysine (%)	0.76	0.76	0.67	0.67	0.65	0.65
Methionine (%)	0.39	0.39	0.33	0.33	0.31	0.31
Calcium (%)	0.43	0.43	0.42	0.42	0.42	0.42
Available Phosphorus (%)	0.38	0.38	0.36	0.36	0.35	0.35
ME (kcal/kg)	2965.04	2965.04	2964.39	2964.39	2964.33	2964.33
Analyzed composition (%)						
Moisture (%)	7.12	7.17	6.99	6.97	6.95	6.93
Crude protein (%)	16.09	16.04	16.64	16.62	17.62	17.65
Crude fibre (%)	11.34	11.32	12.46	12.43	12.85	12.88
Ether extract (%)	3.10	2.99	3.13	3.16	3.19	3.18
Ash (%)	6.20	6.20	6.14	6.13	6.17	6.15
Nitrogen free extract (%)	56.15	56.28	54.64	54.69	53.22	53.21

*RLFCP = Rumen microbial fermented cassava peels; ME = Metabolizable energy; *ME: metabolizable = (37 x % CP) + (81.8 x %FAT) + (35.5 x % NFE) (Pauzenga, 1985).*

The sera which were separated from the plain bottle blood samples were analyzed for total protein (TP), albumin (AL), globulin (GLO), cholesterol (CHO), urea (URE), creatinine (CRE), bilirubin (BIL), aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), amylase (AMY), glucose (GLU) and triglyceride (TRI) with a Reflectron Plus 8C79 (Roche Diagnostic, GonbH Mahnheim, Germany), using commercial kits.

Statistical Analysis: The general linear model (GLM) of SPSS version 20 (SPSS, 2011) was used to analyze the data. The main factors' effects (E and RLFCP) and their interaction were also assessed. The differences between the mean values were separated by Duncan's multiple range test (Duncan, 1955). Level of significance was set at $p<0.05$.

RESULTS

Performance Characteristic: The performance of weaner rabbits fed graded level of RLFCP supplemented with enzyme indicated that the final live weight (FLW), total weight gain (TWG) and daily weight gain (DWG) of the weaner rabbits were significantly affected ($p<0.05$) by the enzyme supplementation levels. The FLW of 1567.67 ± 6.66 g/rabbit, TWG of 1018.17 ± 1.16 g/rabbit and DWG of 18.18 ± 0.02 g/rabbit fed with diet supplemented with 0.35 g/kg enzyme were significantly higher ($p<0.05$) than those fed diets with 0 g/kg enzyme supplementation (Table 3). In the same vein, all the performance characteristics (FLW, TWG, DWG, TFI, DFI and FCR) measured were significantly affected ($p<0.05$) by the level of inclusion of PLFCP. The FLW (1724.74 ± 8.17 g/r vs. 1505.55 ± 8.17 g/r vs. 1433.38 ± 8.17 g/r), TWG (1170.25 ± 1.42 g/r vs. 965.00 ± 1.42 g/r vs. 887.50 ± 1.42 g/r) and DWG (20.91 ± 0.03 g/r/d vs. 17.23 ± 0.03 g/r/d vs. 15.85 ± 0.03 g/r/d) decreased significantly ($p<0.05$) with increased RLFCP inclusion from 0 to 75 to 100% in the diets, while the TFI (3744.08 ± 41.40 g/r vs. 3856.86 ± 41.40 g/r vs. 3938.64 ± 41.40 g/r), DFI (66.86 ± 0.73 g/r/d vs. 68.87 ± 0.73 g/r/d vs. 70.33 ± 0.73 g/r/d) and FCR (3.20 ± 0.04 vs. 3.99 ± 0.04 vs. 4.44 ± 0.04) increased significantly ($p<0.05$) with increased RLFCP in the diets. The enzyme x RLFCP interaction was only significant ($p<0.05$) for TWG and DWG.

Carcass Trait and Relative Organ: Table 4 showed that the slaughtered weights (1384.00 ± 24.47 g vs. 1372.00 ± 24.47 g vs. 1296.50 ± 24.47 g) and skin, head and limb (21.71 ± 0.33 % vs. 20.02 ± 0.33 % vs. 20.38 ± 0.33 %) were significantly affected ($p<0.05$) and these decreased with increased in inclusion levels of RLFCP in the diets.

Table 5 showed that the commercial enzyme supplementation did not significantly affected ($p>0.05$) the relative weights of all the internal organs measured. RLFCP inclusion levels significantly affected the relative weights (% slaughter weight) of the kidney (0.67 ± 0.03 vs. 0.75 ± 0.03 vs. 0.85 ± 0.03), gall bladder (0.07 ± 0.00 vs. 0.07 ± 0.00 vs. 0.06 ± 0.00) and gastrointestinal tract (17.46 ± 0.46 vs. 19.95 ± 0.46 vs. 18.19 ± 0.46) with enzyme x RLFCP being significant ($p<0.05$) for relative weight of gall bladder.

Haematological Indices: Table 6 indicated that while commercial enzyme supplementation did not significantly ($p>0.05$) affect all the haematological parameters measured, RLFCP inclusion level significantly increased ($p<0.05$) the PCV (33.80 ± 0.64% vs. 36.75 ± 0.64% vs. 36.85 ± 0.64%), MCV (65.80 ± 0.52 fl vs. 68.80 ± 0.52 fl vs. 68.38 ± 0.52 fl) and lymphocytes concentrations (1.85 ± 0.16 x 10^9/l vs. 2.53 ± 0.16 x 10^9/l vs. 2.40 ± 0.16 x 10^9/l), with enzyme x RLFCP being significant ($p<0.05$) for RBC.

Serum Metabolites: The serum biochemical profiles considered were not significantly affected ($p>0.05$) by the enzyme supplementation levels and enzyme x RLFCP interaction (Table 7). However, the albumin (49.75 ± 8.01 g/l vs. 56.50 ± 8.01 g/l vs. 53.00 ± 8.01 g/l), globulin (14.00 ± 0.45 g/l vs. 16.00 ± 0.45 g/l vs. 16.50 ± 0.45 g/l), alkaline phosphatase (187.50 ± 8.01µ/l vs. 156.75 ± 8.01µ/l vs. 156.00 ± 8.01 µ/l), amylase (718.75 ± 24.92 µ/l vs. 593.00 ± 24.92 µ/l vs. 733.00 ± 24.92 µ/l)and glucose (41.30 ± 1.78 mg/dl vs. 48.08 ± 1.78 mg/dl vs. 36.83 ± 1.78 mg/dl) were significantly affected ($p<0.05$) by the level of RLFCP in the diets such that replacement level of maize for RLFCP lead to increase in albumin and glucose, alkaline phosphate, while the effect of the replacement levels did not follow a particular trend for amylase and glucose.

DISCUSSION

An earlier reported study gave 50 % RLFCP inclusion level as the optimum in rabbits diets (Oloruntola *et al.*, 2016a). Therefore, possibility of using enzyme supplementation in rabbit diet to improve the performance of weaner rabbits fed diets in which maize was replaced with RLFCP beyond 50% level (75 and 100%) formed the basis for this experiment.

Table 3: Performance of weaner rabbits fed graded levels of rumen liquor fermented cassava peels (RLFCP) supplemented with enzyme (E)

Diets	E (g/kg)	RLFCP (%)	IW	FLW	TWG	DWG	TFI	DFI	FCR
	0.00		544.45 ± 6.48	1541.45 ± 6.66^{b}	997.00 ± 1.16^{b}	17.80 ± 0.02^{b}	3832.83 ± 0.02	68.44 ± 0.60	3.91 ± 0.04
	0.35		549.50 ± 6.48	1567.67 ± 6.66^{a}	1018.17 ± 1.16^{b}	18.18 ± 0.02^{a}	3860.22 ± 0.02	68.93 ± 0.60	3.85 ± 0.04
		0	554.49 ± 7.94	1724.74 ± 8.17^{a}	1170.25 ± 1.42^{a}	20.91 ± 0.03^{a}	3744.08 ± 41.40^{c}	66.86 ± 0.73^{c}	3.20 ± 0.04^{a}
		75	540.55 ± 7.94	1505.55 ± 8.17^{b}	965.00 ± 1.42^{b}	17.23 ± 0.03^{b}	3856.86 ± 41.40^{ab}	68.87 ± 0.73^{ab}	3.99 ± 0.04^{b}
		100	545.88 ± 7.94	1433.38 ± 8.17^{c}	887.50 ± 1.42^{c}	15.85 ± 0.03^{c}	3938.64 ± 41.40^{a}	70.33 ± 0.73^{a}	4.44 ± 0.04^{c}
	Enzyme x RLFCP								
1	**0.00**	**0**	550.62 ± 11.23	1711.12 ± 11.54	1160.50 ± 2.01	20.72 ± 0.04	3748.26 ± 58.55	66.93 ± 1.05	3.23 ± 0.06
2	**0.35**	**0**	558.37 ± 11.23	1160.50 ± 11.54	1180.00 ± 2.01	21.07 ± 0.04	3804.03 ± 58.55	66.78 ± 1.05	3.17 ± 0.06
3	**0.00**	**75**	529.07 ± 11.23	1487.07 ± 11.54	950.00 ± 2.01	17.11 ± 0.04	3946.18 ± 58.55	67.93 ± 1.05	3.97 ± 0.06
4	**0.35**	**75**	552.03 ± 11.23	1524.03 ± 11.54	972.00 ± 2.01	17.36 ± 0.04	3739.88 ± 58.55	69.82 ± 1.05	4.02 ± 0.06
5	**0.00**	**100**	553.67 ± 11.23	1426.17 ± 11.54	872.50 ± 2.01	15.58 ± 0.04	3909.68 ± 58.55	70.47 ± 1.05	4.52 ± 0.06
6	**0.35**	**100**	538.10 ± 11.23	1440.60 ± 11.54	902.50 ± 2.01	16.12 ± 0.04	3931.10 ± 58.55	70.20 ± 1.05	4.36 ± 0.06

Means with different superscripts in the same row are significant (P<0.01, 0.001). IW: Initial live weight (g/rabbit); FLW: Final live weight (g/rabbit); TWG: Total weight gain (g/rabbit); DWG: Daily weight gain (g/rabbit/day); TFI: Total feed intake (g); DFI: Daily feed intake (g/rabbit/day); FCR: Feed conversion ratio

Table 4: Carcass (% slaughter weight) of weaner rabbits fed graded levels of rumen liquor fermented cassava peels (RLFCP) supplemented with enzyme (E)

Diet	E (g/kg)	RLFCP (%)	Slaughtered weight (g)	Dressing %	Skin, head and limb	Hind leg	Fore leg	Breast and rib cage	Loin and abdominal wall	Neck
	0.00		1349.33 ± 19.98	47.68 ± 0.93	20.81 ± 0.27	15.95 ± 0.32	6.54 ± 0.11	9.54 ± 0.16	12.83 ± 0.38	2.82 ± 0.17
	0.35		1352.32 ± 19.98	47.38 ± 0.93	20.59 ± 0.27	15.84 ± 0.32	6.47 ± 0.11	9.46 ± 0.16	12.71 ± 0.38	2.75 ± 0.17
		0	1384.00 ± 24.47^{a}	47.44 ± 1.14	21.71 ± 0.33^{b}	15.59 ± 0.39	6.24 ± 0.14	9.71 ± 0.20	12.99 ± 0.21	3.00 ± 0.21
		75	1372.00 ± 24.47^{ab}	47.57 ± 1.14	20.02 ± 0.33^{a}	15.76 ± 0.39	6.51 ± 0.14	9.42 ± 0.20	12.85 ± 0.21	2.79 ± 0.21
		100	1296.50 ± 24.47^{b}	57.58 ± 1.14	20.38 ± 0.33^{a}	16.33 ± 0.39	6.77 ± 0.14	9.35 ± 0.20	12.46 ± 0.21	2.57 ± .21
	Enzyme x RLFCP									
1	**0.00**	**0**	1372.50 ± 34.61	47.46 ± 1.62	21.71 ± 0.46	15.62 ± 0.56	6.27 ± 0.19	9.75 ± 0.29	13.03 ± 0.66	3.02 ± 0.30
2	**0.35**	**0**	1371.50 ± 34.61	47.42 ± 1.62	21.71 ± 0.46	15.56 ± 0.56	6.21 ± 0.19	9.68 ± 0.29	12.97 ± 0.66	2.98 ± 0.30
3	**0.00**	**75**	1383.49 ± 34.61	47.77 ± 1.62	20.16 ± 0.46	15.86 ± 0.56	6.55 ± 0.19	9.47 ± 0.29	12.93 ± 0.66	2.82 ± 0.30
4	**0.35**	**75**	1385.00± 34.61	47.36 ± 1.62	19.88 ± 0.46	15.67 ± 0.56	6.47 ± 0.19	9.38 ± 0.29	12.77 ± 0.66	2.76 ± 0.30
5	**0.00**	**100**	1292.50± 34.61	47.80 ± 1.62	20.57 ± 0.46	16.38 ± 0.56	6.79 ± 0.19	9.40 ± 0.29	12.54 ± 0.66	2.63 ± 0.30
6	**0.35**	**100**	1300.49± 34.61	47.37 ± 1.62	20.18 ± 0.46	16.29 ± 0.56	6.74 ± 0.19	9.32 ± 0.29	12.40 ± 0.66	2.51 ± 0.30

Means with different superscripts in the same column are significant (p<0.05)

Table 5: Internal organs (% slaughter weight) of weaner rabbits fed graded levels of rumen liquor fermented cassava peels (RLFCP) supplemented with enzyme (E)

Diet	E (g/kg)	RLFCP (%)	Lung	Liver	Kidney	Heart	Pancreas	Gall bladder	Gastrointestinal tract
	0.00		0.56 ± 0.04	2.45 ± 0.12	0.76 ± 0.02	0.23 ± 0.01	0.04 ± 0.00	0.07 ± 0.00	18.59 ± 0.37
	0.35		0.57 ± 0.04	2.49 ± 0.12	0.75 ± 0.02	0.22 ± 0.01	0.04 ± 0.00	0.07 ± 0.00	18.47 ± 0.37
		0	0.60 ± 0.05	2.64 ± 0.14	0.67 ± 0.03^{b}	0.22 ± 0.02	0.05 ± 0.01	0.07 ± 0.00^{a}	17.46 ± 0.46^{b}
		75	0.59 ± 0.05	2.31 ± 0.14	0.75 ± 0.03^{ab}	0.24 ± 0.02	0.04 ± 0.01	0.07 ± 0.00^{a}	19.95 ± 0.46^{a}
		100	0.49 ± 0.05	2.45 ± 0.14	0.85 ± 0.03^{a}	0.21 ± 0.02	0.04 ± 0.01	0.06 ± 0.00^{b}	18.19 ± 0.46^{b}
	Enzyme x RLFCP								
1	**0.00**	**0**	0.57 ± 0.07	2.64 ± 0.21	0.63 ± 0.04	0.21 ± 0.02	0.04 ± 0.01	0.09 ± 0.01	17.50 ± 0.65
2	**0.35**	**0**	0.64 ± 0.07	2.65 ± 0.21	0.72 ± 0.04	0.23 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	17.41 ± 0.65
3	**0.00**	**75**	0.60 ± 0.07	2.37 ± 0.21	0.89 ± 0.04	0.25 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	20.13 ± 0.65
4	**0.35**	**75**	0.58 ± 0.07	2.26 ± 0.21	0.80 ± 0.04	0.23 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	19.78 ± 0.65
5	**0.00**	**100**	0.51 ± 0.07	2.35 ± 0.21	0.77 ± 0.04	0.21 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	18.15 ± 0.65
6	**0.35**	**100**	0.48 ± 0.07	2.55 ± 0.21	0.74 ± 0.04	0.21 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	18.22 ± 0.65

Means with different superscripts in the same column are significant (p<0.05)

Table 6: Haematological indices of weaner rabbits of weaner rabbits fed graded levels of rumen liquor fermented cassava peels (RLFCP) supplemented with enzyme (E)

Diet	E (g/kg)	RLFCP (%)	PCV	RBC	WBC	HBC	MCV	MCH	MCHC	PLA	LYM	MON	GRA
	0.00		35.63 ± 0.52	5.64 ± 0.19	6.65 ± 0.59	12.12 ± 0.18	67.62 ± 0.42	22.65 ± 0.43	33.28 ± 0.38	352.00 ± 28.77	2.15 ± 0.13	0.72 ± 0.06	3.58 ± 0.38
	0.35		35.97 ± 0.52	5.80 ± 0.19	6.66 ± 0.59	12.40 ± 0.18	67.70 ± 0.42	23.65 ± 0.43	33.82 ± 0.38	368.67 ± 28.77	2.37 ± 0.13	0.80 ± 0.06	3.88 ± 0.38
		0	33.80 ± 0.64^{b}	5.80 ± 0.24	6.25 ± 0.73	12.30 ± 0.22	65.80 ± 0.52^{b}	22.50 ± 0.53	34.10 ± 0.46	377.50 ± 0.73	1.85 ± 0.16^{b}	0.70 ± 0.08	3.45 ± 0.47
		70	36.75 ± 0.64^{a}	5.81 ± 0.24	6.40 ± 0.73	12.10 ± 0.22	68.80 ± 0.52^{a}	23.00 ± 0.53	32.70 ± 0.46	331.50 ± 0.73	2.53 ± 0.16^{a}	0.68 ± 0.08	3.15 ± 0.47
		100	36.85 ± 0.64^{a}	5.56 ± 0.24	7.30 ± 0.73	12.38 ± 0.22	68.38 ± 0.52^{a}	23.95 ± 0.53	33.85 ± 0.46	372.00 ± 0.73	2.40 ± 0.16^{a}	0.90 ± 0.08	4.60 ± 0.47
	Enzyme x RLFCP												
1	**0.00**	**0**	34.55 ± 0.91	6.30 ± 0.34	6.75 ± 1.03	12.45 ± 1.03	66.30 ± 0.73	23.00 ± 0.75	33.85 ± 0.66	402.50 ± 49.84	1.85 ± 0.23	0.75 ± 0.11	3.55 ± 0.66
2	**0.35**	**0**	33.05 ± 0.91	5.30 ± 0.34	5.75 ± 1.03	12.15 ± 1.03	65.30 ± 0.73	22.00 ± 0.75	34.35 ± 0.66	352.47 ± 49.84	1.86 ± 0.23	0.65 ± 0.11	3.35 ± 0.66
3	**0.00**	**75**	36.25 ± 0.91	5.32 ± 0.34	6.15 ± 1.03	11.85 ± 1.03	68.30 ± 0.73	22.25 ± 0.75	32.65 ± 0.66	306.51 ± 49.84	2.45 ± 0.23	0.60 ± 0.11	3.10 ± 0.66
4	**0.35**	**75**	37.25 ± 0.91	6.31 ± 0.34	6.64 ± 1.03	12.35 ± 1.03	69.29 ± 0.73	23.75 ± 0.75	32.75 ± 0.66	356.50 ± 49.84	2.60 ± 0.23	0.75 ± 0.11	3.20 ± 0.66
5	**0.00**	**100**	36.10 ± 0.91	5.29 ± 0.34	7.05 ± 1.03	12.05 ± 1.03	68.25 ± 0.73	22.70 ± 0.75	33.36 ± 0.66	347.00 ± 49.84	2.15 ± 0.23	0.80 ± 0.11	4.10 ± 0.66
6	**0.35**	**100**	37.60 ± 0.91	5.78 ± 0.34	7.55 ± 1.03	12.70 ± 1.03	68.49 ± 0.73	25.20 ± 0.75	34.35 ± 0.66	398.00 ± 49.84	2.65 ± 0.23	1.00 ± 0.11	5.10 ± 0.66

Means with different superscripts in the same column are significantly different (p<0.05), WBC: White blood cells (x10^{9}/l); LYM: Lymphocytes (x10^{9}/l); MON: Monocytes (x10^{9}/l); GRA: Granulocytes (x10^{9}/l); RBC: Red blood cells (x10^{12}/l); HBC: Haemoglobin conc. (g/dl); PCV: Packed cell volume (%), MCV: Mean cell volume (fl); MCH: Mean cell haemoglobin (pg); MCHC: Mean cell haemoglobin concentration (g/dl); PLA: Platelets (10^{9}/l)

Table 7: Serum biochemical profiles of weaner rabbits fed graded levels of rumen liquor fermented cassava peels (RLFCP) supplemented with enzyme (E)

Diet	E (g/kg)	RLFCP (%)	TP	AL	GLO	HO	URE	CRE	BIL
	0.00		61.67 ± 2.49	54.00 ± 0.15	43.63 ± 0.23	121.00 ± 4.87	44.08 ± 2.61	1.38 ± 0.08	1.44 ± 0.16
	0.35		64.17 ± 2.49	52.17 ± 0.83	40.50 ± 0.37	114.17 ± 4.87	42.95 ± 2.61	1.42 ± 0.08	1.50 ± 0.16
		0	60.75 ± 3.05	49.75 ± 8.01^{b}	14.00 ± 0.45^{b}	104.50 ± 5.97	39.50 ± 3.19	1.27 ± 0.10	1.44 ± 0.20
		75	61.50 ± 3.05	56.50 ± 8.01^{a}	16.00 ± 0.45^{a}	124.50 ± 5.97	46.70 ± 3.19	1.51 ± 0.10	1.41 ± 0.20
		100	66.00 ± 3.05	53.00 ± 8.01^{ab}	16.50 ± 0.45^{a}	123.75 ± 5.97	44.35 ± 3.19	1.42 ± 0.10	1.57 ± 0.20
	E x RLFCP								
1	**0.00**	**0**	61.50 ± 4.32	49.00 ± 1.44	14.50 ± 0.65	107.00 ± 8.44	40.50 ± 4.52	1.17 ± 0.14	1.48 ± 0.28
2	**0.35**	**0**	60.00 ± 4.32	50.50 ± 1.44	13.50 ± 0.65	102.50 ± 8.44	38.52 ± 4.52	1.36 ± 0.14	1.39 ± 0.28
3	**0.00**	**75**	62.00 ± 4.32	57.51 ± 1.44	16.46 ± 0.65	129.50 ± 8.44	46.65 ± 4.52	1.56 ± 0.14	1.35 ± 0.28
4	**0.35**	**75**	61.00 ± 4.32	55.50 ± 1.44	15.49 ± 0.65	119.50 ± 8.44	47.73 ± 4.52	1.46 ± 0.14	1.46 ± 0.28
5	**0.00**	**100**	61.50 ± 4.32	55.50 ± 1.44	16.50 ± 0.65	126.46 ± 8.44	45.10 ± 4.52	1.40 ± 0.14	1.49 ± 0.28
6	**0.35**	**100**	71.48 ± 4.32	50.53 ± 1.44	16.45 ± 0.65	121.00 ± 8.44	43.60 ± 4.52	1.45 ± 0.14	1.66 ± 0.28
Diet	**E (g/kg)**	**RLFCP (%)**		**AST**	**ALT**	**ALP**	**AMY**	**GLU**	**TRI**
	0.00			136.28 ± 17.45	68.80 ± 7.27	162.00 ± 6.54	685.00 ± 20.35	43.63 ± 1.45	203.50 ± 21.38
	0.35			153.50 ± 17.45	67.00 ± 7.26	171.50 ± 6.54	678.17 ± 20.35	40.50 ± 1.45	211.00 ± 21.38
		0		164.50 ± 21.37	65.63 ± 8.89	187.50 ± 8.01^{a}	718.75 ± 24.92^{a}	41.30 ± 1.78^{ab}	200.25 ± 26.18
		75		145.40 ± 21.37	81.55 ± 8.89	156.75 ± 8.01^{ab}	593.00 ± 24.92^{b}	48.08 ± 1.78^{a}	228.25 ± 26.18
		100		124.78 ± 21.37	56.53 ± 8.89	156.00 ± 8.01^{ab}	733.00 ± 24.92^{a}	36.83 ± 1.78^{ab}	193.00 ± 26.18
	E x RLFCP								
1	**0.00**	**0**		163.00 ± 30.23	65.70 ± 12.58	185.00 ± 11.33	717.50 ± 35.24	42.30 ± 2.52	193.00 ± 37.03
2	**0.35**	**0**		166.00 ± 30.23	65.70 ± 12.58	190.00 ± 11.33	720.00 ± 35.24	40.30 ± 2.52	207.50 ± 37.03
3	**0.00**	**75**		150.40 ± 30.23	86.55 ± 12.58	157.50 ± 11.33	565.00 ± 35.24	48.35 ± 2.52	229.00 ± 37.03
4	**0.35**	**75**		140.40 ± 30.23	76.56 ± 12.58	156.00 ± 11.33	621.00 ± 35.24	47.80 ± 2.52	227.50 ± 37.03
5	**0.00**	**100**		95.45 ± 30.23	54.15 ± 12.58	143.50 ± 11.33	772.50 ± 35.24	40.25 ± 2.52	188.51 ± 37.03
6	**0.35**	**100**		154.10 ± 30.23	58.90 ± 12.58	168.50 ± 11.33	693.50 ± 35.24	33.40 ± 2.52	198.00 ± 37.03

Means with different superscripts in the same column are significantly different (P<0.05). TP: Total protein (g/l); AL: Albumin (g/l); GLO: Globulin (g/l); CHO: Cholesterol (mg/dl); URE: Urea (mg/dl); CRE: Creatinine (mg/dl); BIL: Bilirubin (µ/l); SGOT: Serum glutamic oxalate acetic transaminase (µ/l); SGPT: Serum glutamic pyruvic transaminase (µ/l); ALP: Alkaline phosphate (µ/l); AMY: Amylase (µ/l), GLU: Glucose (mg/dl); TRI: Triglyceride (mg/dl).

Nutritional values of cereal grains and their by-products in some species of monogastrics diets had been improved by enzyme supplementation (Bedford, 2000). In this study, the enzyme supplementation led to improvement in FLW, TWG and DWG of the weaner rabbits. This suggested that the enzyme supplementation improved the nutrient availability for the weaner rabbits. Enzymes supplementation had been reported to improve the nutritional value of feed ingredients, increasing the efficiency of digestion and helps break down anti-nutritional factors in feed that can interfere with normal digestion, reduce feed efficiency and caused digestive upsets (Barletta, 2011). Feed enzymes are used to increase the availability of starch, protein, amino acids and minerals such as phosphorus and calcium from feed ingredients (Barletta, 2011). This report agreed with the report of Gutierrez *et al.* (2002) that enzyme supplementation led to improvement in fibre digestibility and Eiben *et al* (2004) who reported an improvement in feed conversion ratio and reduced mortality rate in weaner rabbits fed cellulase supplemented diet. Igbasan *et al.* (1997) reported improved growth rate in broiler chickens fed diets containing peas with pectinase and alpha-galactosidase supplementation. El-Sagheer and Hassanein (2014) reported improved body weight, body weight gain and feed conversion ratio of growing female rabbits fed commercial diet supplemented with commercial enzyme mixture. Recently, Ayodele *et al.* (2016) reported improved crude protein and crude fibre digestibility in rabbits fed enzyme supplemented diets. The report of this study disagreed with the report of Garcia-Ruiz *et al*. (2006) and Falcao-e-Cunha *et al.* (2007) who could not detect any significant effect of enzymes on rabbit's performance. Decrease in the FLW, TWG, DWG and FCR of the weaner rabbits with increase in RLFCP inclusion level may be as a result of possible increase in the level of anti-nutrients (hydrogen cyanide, phytate, tannins) in the diets as the RLFCP inclusion increased from 0 to 100 %. The presence of these anti-nutrients in diets has been associated with reduced animal performance (Alawa and Amadi, 1991; Adegbola and Oduozo, 1992). The progressive increase in feed intake of rabbits in association with increase in the replacement level of RLFCP may be connected with gradual increase in fibre content associated with increase in replacement of RLFCP across the diets. Osakwe and Nwosu (2008) reported similar result that feed intake of weaner rabbits increased with increase of dietary fibre because of increase in replacement of maize by cassava peels.

Growing rabbits naturally consume sufficient feed to meet their energy requirement (Xiccato and Trocino, 2010). The interaction between enzyme and levels of RLFCP inclusion on total weight gain and daily weight gain in this study suggested that they play important role in growth of the weaner rabbits. The differences in the slaughtered weight as the replacement of RLFCP increased from 0 to 100% could be attributed to variations in the live weights of the experimental rabbits. This difference in slaughtered weight agreed with Retore *et al.* (2008) who fed diets of varying quality to growing rabbits. The slaughtered weight ranges of 1920 to 2190 g and 1594.57 to 1792.85 g reported by Retore *et al.* (2008) and Olafadehan (2011) respectively were higher than values obtained in this study. However, this variation may be due to differences in the nutrition, age at slaughter and breed type among others. The percentage skin, head and limb in this study was similar to 21.17 % reported by Yalcin *et al.* (2006). The decrease in percentage skin, head and limb of weaner rabbits as the RLFCP replacement level increased in this study lead to decrease in the slaughtered weight of the experimental rabbits. Butcher *et al.* (1983) had earlier reported that relative weights of external offal such as pelt tend to increase as slaughtered weight of rabbits increased. Furthermore, the variation in pelt percentage is possibly due to different levels of fat deposition due to dietary treatments and slaughter weight (Olafadehan, 2011), and in this study, it may be due to increase in replacement level of RLFCP in the weaner rabbits' diets. The result of this study indicated that out of the entire internal organ weights measured; only the kidney, bile and gastrointestinal tract were affected by the

RLFCP inclusion levels. Furthermore, bile weight was also affected by enzyme x RLFCP interaction. The kidneys perform a range of vital functions such as: removing wastes and water from the blood, balancing chemicals in the body, releasing hormones, helping in control of blood pressure, helping to produce red blood cells and producing vitamin D, which keep the bone strong and healthy (Ganong, 2001). The inclusion of RLFCP beyond 75% in the rabbits' diet precipitated significant increase in relative weight of the kidneys of the weaner rabbits. This agreed with the report of Ogunsipe *et al.* (2014), who observed higher weights of kidneys of rabbits fed 75 and 100% sorghum offal-based diets. Gall bladder is the storage sac for bile. The bile is a greenish yellow, thick, sticky fluid which consists of bile salts, electrolytes, bile pigments, cholesterol and fats. The solubility and re-absorption of cholesterol, fat and fat soluble vitamins is dependent on bile. Bile also aids excretion of bilirubin as waste products of destroyed cells, excretion of drugs and other waste products (Carey and Robins, 1987; (Ganong, 2001). In this study, the increase replacement of maize above 75% by RLFCP caused significant decrease in weight of weaner rabbits' gall bladder. The changes in the weights of these vital organs (bile and kidney) may be the effect of their extra activities in detoxifying and excreting the possible anitinutrients in the diets (Dutta *et al.,* 1986). Although, RLFCP had significant influence on the weight of gastrointestinal tract, this did not follow a definite trend.

Blood profiles have been a useful indicator to detect feed toxicity, monitor feed quality and establish the health status of the animals when they are subjected to dietary treatments that could cause major or minor deviation from their normal physiological condition (Aro and Akinmoegun, 2012; Oloruntola *et al.,* 2016b). In this study the PCV, MCV and lymphocytes of the rabbits were increased due to the replacement of maize with RLFCP. PCV is the percentage of blood volume taken up by red blood cells, and MCV increased with increase in the replacement level of maize with RLFCP, particularly at 75 % RLFCP but slightly decreased in animal fed 100 % RLFCP diet. However, the PCV and MCV ranges recorded in this study were within the normal range of 33 – 50 % for PCV and 50 – 75 fl for MCV reported by Latimer *et al.* (2003) and Flecknell (2000) respectively. This further indicated that the dietary treatments positively affected the red blood cell formation and does not tamper with the normal health of the rabbits. The significant interactive effect ($p<0.05$) of enzyme and RLFCP on red blood cell (RBC) indicated the importance of these dietary variables in red blood cell formation. Lymphocytes are type of white blood cells, whose importance in immune system involved the elimination of threats to the body by recognizing the foreign material and producing the chemicals to destroy the foreign material (Oloruntola *et al.* 2016b). In this study, the lymphocytes increased with increased inclusion of RLFCP. Increase in lymphocytes may lead to lymphocytosis due to physiological leukocytosis, young age and chronic infections (Peter and Susan, 1991). Although, in this study, case of lymphocytosis was not noticed, may be because the observed lymphocyte ranges were within the normal range of 1.6 – 10.6 x 10^9/l and 2.0 – 20.0 x 10^9/l reported by Latimer *et al.* (2003) and Flecknell (2000) respectively.

Enzymes are protein within cells that catalyze the reactions for which the cells are responsible. Therefore, an increased enzyme activity in serum implies a problem in cell population from which the enzyme is derived (McCurnin and Basset, 2005). The adequacy of the dietary treatment used in this study was responsible for the stability of total protein, cholesterol, urea, creatinine, bilirubin, serum glutamic oxalate acetic transaminase, serum glutamic pyruvic transaminase and triglyceride among the various rabbits fed varied dietary treatments, as there were no significant effects observed in the levels of these biochemical. Albumin, which is the most abundant plasma protein is synthesize in the liver and essential in the regulation of osmotic pressure and transportation of unconjugated bilirubin and fatty acids (Rothschild *et al*., 1988; McCurnin and Basset, 2005). Albumin level is influence by nutrition and liver condition as its synthesis diminishes during fasting and malnutrition

(Mohammed *et al.,* 2010). In the present study, the albumin levels significantly differed and the albumin ranges recorded for rabbits on diets with 75 and 100 % maize replaced with RLFCP were outside the normal range (33 – 50 g/l) reported by Flecknell (2000). This suggested that the replacement of maize with RLFCP up to 75 % might precipitate hazard to the health of the rabbits. Increased albumin concentrations may be because of haemoconcentration due to dehydration, shock and lipaemia (increased cholesterol in the blood) (Peter and Susan, 1991). However, increased albumin in this study may not be due to lipaemia as the values of cholesterol were within the normal range and not significantly affected ($p>0.05$) by increased in RLFCP level.

Globulin plays a major role in the transportation of substance such as thyroxin, lipids, copper and iron (McCurnin and Basset, 2005). Furthermore, globulins are very important antibodies and component of the complement system (McCurnin and Basset, 2005). In this current study, there were increased globulin values associated with rabbits fed diets with 75 and 100 % maize replaced with RLFCP. Increased globulin values have been linked with haemoconcentration due to shock, dehydration and increased alpha globulins because of fever, trauma or infection. However, the fact that none of these symptoms were observed in the rabbits, further support the suitability of RLFCP for rabbits' feeding. In addition, the range values for globulin in this study were within the normal range values (15 – 27 g/l) (Flecknell, 2000).

The ALP is enzyme found primarily in the liver and bone is measure to determine the occurrence of liver or bone disease. Increased in ALP may be due to congestion or obstruction of biliary tract, while decreased value may be due to deficiencies of zinc, vitamin C and B_6, excess vitamin D intake and malnutrition with low protein assimilation. The ALP values were comparable across the various dietary treatments, although numerical reduction in values of the enzyme occurred across RLFCP replacement levels.

However, the ALP ranges in this study were within the normal range (100 – 400 µ/l) for rabbits (Flecknell, 2000). This result overruled the possibilities of deficiencies of vital minerals and vitamin D or occurrence of low protein assimilation which are the major symptoms associated with abnormally increased ALP. In addition, decreased ALP values were not clinically significant and had similarly been reported (Peter and Susan, 1991).

Amylase is an enzyme produced by pancrease and salivary gland. Normally, small amount of this enzyme is present in the blood but increased amount may be released into the blood when the pancrease is injured or in cases of pancreatitis and blockage of the duct that carries amylase and other substances from pancrease to the small intestine. The effect of RLFCP replacement for maize on amylase in this study did not follow any definite pattern. Although, the amylase level range values were higher than normal range values 200 – 500 µ/l and 167 – 315 µ/l reported by Flecknell (2000) and Latimer *et al.* (2003) respectively suggested possible unset of pancreatitis. However, this possibility may not be true as there was not significant effect of the dietary treatments on the relative weight of the pancreas of the rabbits. Thus, the variation in amylase level may not have negative effect on the health status of the rabbits.

Glucose is a chemical constituent of blood and important source of energy for the cells that makes up the muscle and tissue; although too much of glucose leads to serious health problems (McCurnin and Basset, 2005). The glucose level in this study did not follow definite trend with levels of replacement of maize with RLFCP. However, the glucose level ranges observed in this study were within normal range values (75 – 155 mg/dl) for rabbits (Flecknell, 2000; Latimer *et al.,* 2003).

Conclusion: Multi-enzyme supplementation as used in this study at dosage of 0.35 g/kg improved the weight gain of the rabbits when fed with RLFCP based diets.

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