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INFLUENCE OF THE PROBIOTIC, RE 3 ON NUTRITIONAL PERFORMANCE, HEMATOLOGICAL, IMMUNE STATUS AND CARCASS CHARACTERISTICS OF RABBIT REARED UNDER TROPICAL CONDITIONS

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ABSTRACT: *Thirty-six heterogenous population of California White, New Zealand White and Chinchilla weaner cross-bred rabbits of mean weight of 550 g were randomly assigned to four treatments of nine animals per treatment. The study was structured in such a way that there were two controls i. e. To- (treatment group without any additive in the basal diet) and To+ (treatment group treated with coccidiostat prior to commencement of feeding trial and fed the basal diet). The test treatment groups consisted of T_1 (supplemented with 1.0 ml RE 3 per kg feed) and T_2 (supplemented with 1.5 ml RE 3 per kg feed). The feeding trial lasted for a period of four months after which nutritional indices, hematological, immune function as well as carcass characteristics of the rabbits were assessed. The results of the lymphoid organ and indices showed that all the rabbits had similar immune response regardless of treatment. That was to imply that the immune function and status of all the rabbits seemed to be at the same level regardless of the presence or absence of RE 3. Furthermore, RE 3 neither influenced the growth nor the feed intake while feed conversion efficiency of rabbits fed 1.0 ml RE 3 per kg feed (T_1) demonstrated significant ($P<0.05$) improvement. Rabbits fed treatment T_1 also showed higher significant ($P<0.05$) serum levels of white blood cells and lymphocytes compared to those fed the other treatments. Also, RE 3 as a probiotic did not influence live weight, full stomach, full gastrointestinal and carcass length. It, however, caused significant ($P<0.05$) changes in the warm and chilled dress weights relative to all the others fed the other treatments.*

Key words: *Rabbit, Probiotic, Immune status, Hematology, Carcass, Nutritional profile, Tropical conditions*

ORIGINAL ARTICLE

INTRODUCTION

As a result of the high reproductive capacity of rabbits (*Oryctolagus cunniculus*), it is seen as a highly profitable animal agricultural venture as well as a valuable model for a variety of studies particularly immune and toxicological research according to Pűschel et al. (2010). Nonetheless, they are reportedly more prone to viral, bacterial, fungal and parasitic diseases such as pasteurellosis and coccidiosis (Mailafia et al., 2010). The ability of the natural intestinal complex and dynamic microbial ecosystem to fight intestinal infections is reportedly not always effective and supplementation with probiotic bacteria has proven to support as well as aid treating infections at that level (Corcionivoschi et al., 2010). According to the EEC directive 70/524, several microorganisms (*Bacillus cereus*, *Bacillus substilis*, *Enterococcus faecium*, *Lactobacillus farciminis,* etc) have been authorized as new additive for feedstuffs (Auclair, 2011) and all these strains have been reported to demonstrate positive influence on different animal models namely broiler chicken, beef cattle, dairy cow, piglets, sows and rabbits.

Feed additives are a group of feed ingredients that can elicit a desired animal response in a non-nutrient role such as pH shift, growth or metabolic modifier (Hutjens, 1991). However, they are not a requirement or guarantee for high productivity or profitability. Studies carried out so far indicate that commercial probiotics offer increased specific micro-flora, increased productive parameters, enhance better sanitary conditions, maintain a balance and multiplication of the beneficial microbial population in the gastro-intestinal tract (GIT), alter pre-existing intestinal flora so as to provide an advantage to the host as well as shape the immune systems etc. (Corcionivoschi et al., 2010).

Investigations conducted on the probiotic, RE 3 in Ghana using different animal models have generated a myriad of responses in the form of growth rate improvement, efficiency of feed utilization in pigs and poultry, superior egg production and characteristics as well as lowered mortality in laying birds, weight gain and delayed

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weight loss under feed-stress conditions in sheep (Osei et al., 2008; Okai, 2010; Oppong-Anane, 2009). Evaluation of the efficacy of RE 3 on rabbit under this tropical environment is yet to be undertaken.

This study therefore, set out to assess the influence of RE 3 on the nutritional and carcass characteristics, hematological indices as well as immune profile of rabbits raised under the Ghanaian tropical conditions.

MATERIALS AND METHODS

Background to the Study Location

The work was conducted at the rabbitory of the CSIR-Animal Research Institute's Frafraha station, Accra which is located at the coastal savannah zone of Ghana. The meteorological conditions of the site is such that it has an annual rainfall of about 730 mm which is characterized by two rainy season patterns i. e. May – mid July (major) and mid-August – October (minor). The average temperature ranged between 24.7 (August) and 28 ^{o}C (March). The relative humidity generally stand at 65% (mid-afternoon) and 95% (night time) while wind speed usually ranges between 8 and 16 km/h (http://www.ama.ghanadistricts.gov.gh).

Experimental Design and Feeding Trial

Thirty-six heterogenous population of California White, New Zealand White and Chinchilla weaner cross-bred rabbits of 550 g average weight and aged 6 weeks were obtained from three Ministry of Food and Agriculture certified rabbit farms in Accra, Ghana. They were acclimatized on the Animal Research Institute rabbitory where the study was conducted for two weeks prior to commencement. During that period, all the rabbits were subjected to internal and external parasitic control treatment and they were fed on a compounded feed (Table 1).

They were all treated with internal and external parasite control during this period and fed compounded feed (Table 1). Four treatment groups comprising nine rabbits per treatment in a completely randomized block design format were made. Sex and weight of the animals were factored into the groupings. The treatments were made up of To- (control diet without any additive), To+ (positive control diet which had rabbits treated with the coccidiostat, Vitacox at a ratio of 1:1 continuously for three days followed by a booster on days 5 and 6 but fed diet with any additive inclusion), T_1 (1.0 ml RE 3 per kg compounded feed) and T_2 (1.5 ml RE 3 per kg compounded feed). The feeding trial covered four months and all the rabbits were given feed at 6% of their respective body weights and these were adjusted at weekly intervals. Water was freely provided.

Table 1 - Composition of rabbit diet

Ingredients	Percentage (%)
Maize	51
Soybean meal	14
Wheat bran	32
Dicalcium phosphate	1.20
Oyster shells	1.00
Iodated salt	0.35
Lysine	0.10
Methionine	0.10
*Premix	0.25
Total	100.00
Calculated analysis	
ME (MJ/Kg)	10.30
Crude Protein (%)	16.04
Crude fat (%)	3.39
Crude fibre (%)	4.90
Lysine (%)	0.70
Met + Cystine (%)	0.54
Calcium (%)	0.73
Available phosphorous (%)	0.41
Sodium (%)	0.17

*Premix: Vit. A - 12,000,000 IU; Vit. E - 15000 mg; Vit.B_8 - 1500 mg; Niacin - 30,000 mg; Vit.B_6 - 1500 mg; Vit. D_3 - 4500,000 mg; Vit. K_3 - 3,000 mg; Pantothenic acid - 12000 mg; Vit.B_{12}, - 10,000 mg; Vit. B_2 - 6000 mg; Folic acid -,800 mg, Iron - 60,000 mg; Copper - 75,000 mg; Iodine - 750 mg; Manganese - 130,000 mg; Zinc - 70,000 mg; Selenium - 300mg; Calcium - 17.50%, Lysine - 1,330 mg; Methionine - 1,075 mg; ß-Corotenic acid - 350 mg

Data Collection and laboratory Analysis

As part of the monitoring and evaluating the efficacy of RE 3 as a growth promoting agent in rabbit production, weekly live weight, daily feed intake, daily leftover feed, morbidity and mortality were closely monitored and properly documented. Physical as well as behavioral changes in the rabbits throughout the course of the feeding trial were monitored and documented. At the end of the feeding trial, three rabbits from each treatment group were randomly selected and euthanized. 5 ml of blood was collected by cardiac puncture into labeled, sterile bottles containing EDTA (anti-coagulant) and used to determine the hematological parameters using an automated analyzer, Sysmex KX-210, Sysmex Corporation, Japan.

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The euthanized rabbits were then, weighed, sacrificed and eviscerated. The gastro-intestinal tract (GIT) of the rabbits was also removed and the empty, warm and chilled carcass weights determined and recorded. Also, the full stomach, brain, heart, liver, lung, kidney, testis, tongue, trachea and carcass length were similarly treated and the information generated recorded. The spleen was excised, blotted dry and weighed. The spleen index which contributed to the measure of the immune function of the animals was determined by the method as described by Lu et al. (1996).

Statistical analysis

The data collected were subjected to the one-way-analysis of variance (ANOVA) and the differences between the means assayed by the least significant differences using the Genstat statistical software (Genstat Statistical Package, 2008).

RESULTS AND DISCUSSION

Influence of RE 3 on Nutritional Parameters of rabbits

The nutritional performance of rabbits in response to the introduction of Re 3 as additive in diets is presented in Table 2. The results indicated that no significant ($P>0.05$) differences were observed in the daily feed intake values for all the treatments, neither were there any differences between the final body weights and the average daily weight gains of the rabbits in the four dietary treatment groups ($P>0.05$). However the average daily weight gains on T_1 tended to be superior to the other treatments. This is in agreement with the assertion that the administration of probiotic to fattening rabbits improves growth performance characteristics (Kritas et al., 2008). Efficiency of feed utilization was only significantly different ($P<0.05$) between T_1 and T_2, due possibly to the fact that the rabbits on T_1 made better use of the ingested feed than those on the other treatment groups. That is even though rabbits fed all the treatments had statistically similar ($P>0.05$) feed intake, average daily gain and total weight responses, those fed treatment T1 demonstrated statistically superior feed conversion efficiency ($P<0.05$).

Table 2 - Effect of RE3 on feed intake, live weight changes, feed conversion ratio and health of rabbits

Treatment	Initial Weight	Final Weight	Total Weight Gain	ADG	Total Feed Intake	Av. Daily Feed Intake	FCE	Mortality
To+	1,440	2,720	1,670	19.42	8,458	98.4	5.062^{b}	2 (22.22%)
To-	1,044	2,830	1,730	20.12	8,927	103.8	5.225^{ab}	1 (11.11%)
T1	1,033	2,765	1,755	20.41	8,201	95.4	4.739^{b}	0
T2	1,056	2,778	1,600	18.60	9,079	105.6	5.750^{a}	3
L.S.D.	–	471.0	349.7	0.004	1.340	15.58	0.680	–

Means in a column with similar or no superscripts are not significantly different ($P>0.05$). ADG = Average Daily Gain; FCE = Feed Conversion Efficiency; Av. Daily Feed Intake = Average Daily Feed Intake

Probiotics according to Metzler et al. (2005) are used as a nutritional technique to support host organisms during difficult physiological periods, attenuation of technological stress or prevent and combat diarrheal syndromes. Therefore, its usage does not seem to be focused directly on enhancing nutritional performance of animals. However, specific examples available literature indicate otherwise relative to some animal models such as weaned pigs (improved body weight gain and growth performance), birds (improved performance and productivity – growth, increases in egg production and feed conversion), cattle (increased feed intake and body weight and lambs (improvement in growth performance and meat production) [Philips et al., 1985; Lema et al., 2001; Baum et al., 2002; Konstantinov et al., 2004].

Further, the results also demonstrated that there was statistical insignificant differences ($P>0.05$) in the rate of mortality among the rabbits on all the treatment groups. This does not seem to corroborate findings of Kritas et al. (2008) who asserted that even when the health status of rabbits were satisfactory, mortality was significantly reduced after treatment with probiotics during the growing period.

Effect of RE 3 on hematological parameters of rabbits

Blood examination reportedly gives the opportunity to investigate the presence of several metabolites as well as other constituents and thus help in detecting conditions of stress, which could be nutritional, environmental or physical and that physiological parameters (hormones, heart rate, immune reactions) when considered in relation with other parameters (behavior, morbidity, etc.) can be used as a welfare indicator (Aderemi, 2004; Hoy and Verga, 2007; Archetti et al., 2008). Table 3 presents the hematological profile of rabbits fed RE 3 at inclusion rates of 1.0 and 1.5 ml per kg feed (T_1 and T_2 respectively). The results revealed that there were no treatment effects in the blood profile of the animals and that the values obtained in this study were within the normal references for rabbits (Ross et al., 1979; Mitruka and Rawnsle, 1997; Ahamefule et al., 2008, www.medirabbit.com). RE 3 as a probiotic can thus be said to sustain the normal hematopoietic function of rabbits at the inclusion rates of 1.0 to 1.5 ml per kg feed as no significant differences ($P>0.05$) were established among all the treatment groups. This notwithstanding, the study showed a significantly ($P<0.05$) higher levels of WBC and lymphocytes ($\times 10^3/\mu l$) for T_1 whilst the other treatments had values that fell within the reference range ($5 – 13 \times 10^3/\mu l$) for rabbits (http:www.medirabbit.com).

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Table 3 - Effect of RE 3 on hematological parameters of rabbits direct- fed for 4 months

Treatment	Hb (%)	HCT (g/dl)	RBC ($\times 10^6$/µl)	RDW_SD (fl)	MCH (pg)	MCHC (g/dl)	MCV (fl)	MPV (fl)	PDW (fl)	PLT ($\times 10^3$/µl)	P_LCR (%)	WBC ($\times 10^6$/µl)	LYM ($\times 10^3$/µl)	LYM (%)
To+	12.87	40.70	6.12	30.03	20.90	31.67	66.10	6.73	7.93	365.00	6.33	6.97^{b}	3.73^{c}	53.4
To-	13.43	43.20	6.32	31.63	21.33	31.07	68.70	7.37	8.07	368.00	4.07	11.09^{a}	7.17^{ab}	65.7
T_1	13.80	44.20	6.48	30.70	21.30	31.23	68.13	7.07	8.17	320.00	4.43	13.33^{a}	8.37^{a}	63.0
T_2	12.10	38.20	5.59	31.83	21.63	31.67	68.33	6.53	7.40	445.00	5.53	11.93^{b}	6.47^{b}	55.5
L.S.D.	2.407	7.14	1.358	4.371	1.81	0.675	6.41	1.90	2.01	361.00	3.649	3.36	1.462	13.9

Means in a column with similar or no superscripts are not significantly different (P>0.05). Hb=Hemoglobin concentration; HCT=Hematocrit; RBC=Red blood cell; MCH=Mean cell hemoglobin; MCHC=Mean cell hemoglobin concentration; MCV=Mean cell volume; MPV=Mean platelets volume; PDW= platelets distribution width; PLT=Platelets; P_LCR=platelets large cell ratio; WBC= White blood cell; LYM= Lymphocytes

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High WBC count has been reported to be usually associated with microbial infection or the presence of foreign bodies or antigens in the circulatory system (Ahamefule et al., 2006). None of these scenarios could be the basis for the observed comparatively high WBC count as well as lymphocytes in rabbits fed treatment T_1. This could be the basis for stimulating and boosting the immune status of the rabbits on that treatment. This may, however, need confirmation from actual measurements of the specific immune responses (serum immunoglobulins such as IgM, etc.).

From the statistically insignificant variations in mortality rates observed among all the treatment groups coupled by relatively higher levels of WBC and lymphocytes, it would not be out of place to attest to the comparative effectiveness of RE 3 in maintaining the natural defense mechanism of rabbits introduced the product as part of the diet regimen. A similar observation was made in relation to pigs given 1.5 ml RE 3/kg feed (Owusu Amoah, 2010).

Effect of RE 3 on immune function of rabbits

Immune organs are those whose functions help maintain the normal immune status of the bodies of animals (Feng et al., 2007). In this regards, the weight of lymphoid organ as well as their indices commonly serve as a measure of the immune status (Pope, 1991). The results of the present work as presented in Table 4 showed that there were no significant (P>0.05) differences in the spleen indices as well as weights of spleen of the rabbits in the various treatment groups. Based on this, it could be inferred that the use of RE 3, at the inclusion rate of 1.0 and 1.5 ml/kg feed, elicited similar immune responses just as the coccidiostat-treated counterparts (To+).

Table 4 - The impact of RE3 on the immune status of rabbit

Treatment	Weight of Spleen (g)	Spleen Index
T_{0+}	1.67	0.60
T_{0-}	1.67	0.64
T_1	1.33	0.45
T_2	2.00	0.52
L.S.D.	1.88	0.77

The impact of RE 3 on the immune response of rabbits introduced to it does not seem to conform to the general impression about probiotics in terms of immune stimulation. Probiotic micro-organisms in the gut reportedly have the capacity to stimulate the immune system either by migrating through the gut wall as viable cells which multiply to a limited extent or antigens released by the dead organisms get absorbed and stimulate the immune system directly (www.albertaclassic.net/probiotics.php).

Influence of RE 3 on carcass characteristics and some vital organs of rabbits

Most of the carcass indices such as the full stomach, brain, liver, lungs, kidneys, heart, testicles, tongue and trachea and carcass length of the rabbits were similar in weight among all the dietary treatments (Table 5). This may imply that RE 3 does not induce any toxicological influence that could cause hypertrophy of organs or the level of RE 3 fed might not be sufficiently large enough to induce such a response. Histopathological examination of certain key organs of these rabbits as well as blood chemistry did not lend credence to organ injury or damage as RE 3 introduction as additive. The chilled carcass weight of rabbits fed 1.0 ml RE 3 per kg feed (T1), however, differed significantly (P<0.05) from those treated with coccidiostat (To+). This weight was also higher than that of those fed a higher dose of RE 3 (T2). Similar observations were made by Apgar et al. (1993) and Okai (2010).

Table 5 - Carcass weight of some organs of rabbits fed RE3 for a period of 4 months (g)

Trt	Live Wt	Warm Dressed Wt	Chilled dressed wt	Full stom	Full GIT	Brain	Heart	Lung	Liver	Kidney	testis	Ton & Tra	Carcass length/ cm
To+	2,700	1,697^{b}	1,576^{bc}	91.30	441	7.67	7.33	13.70	67.0	13.00	6.83	11.67	29.69
To-	2,767	1,740^{a}	1,610^{ab}	115.30	411	7.67	8.00	17.70	77.0	12.67	8.58	11.67	29.83
T1	2,833	1,781^{a}	1,650^{a}	121.30	479	6.67	9.67	11.00	77.3	13.67	7.83	12.67	30.05
T2	2,667	1,677^{b}	1,560^{c}	87.70	392	7.33	8.33	14.70	74.7	13.67	7.08	11.33	29.84
L.S.D.	570.0	41.80	41.70	57.99	210.5	2.88	3.81	11.29	14.72	3.21	6.21	2.514	1.999

Means in a column with similar or no superscripts are not significantly different (P>0.05). Trt= Treatment; Full Stom = Full Stomach; Ton & Tra= Tongue and Trachea; GIT= Gastrointestinal Tract; Warm Dress Wt= Warm dressing Weight; Chilled Dress Wt == Chilled Dressing Weight

CONCLUSION

The study revealed that the use of this probiotic, RE 3 could enhance the concentration of white blood cells and lymphocyte particularly at the inclusion rate of 1.0 ml per kg feed. Furthermore, weight gain, feed intake and average daily gain were not influenced by the inclusion of RE 3. However, feed conversion efficiency (FCE) of rabbits supplemented with 1.0 ml per kg feed (T1) tended to be better than those provided 1.5 ml per kg feed (T2) which is suggestive of the fact that treatment T1 was a better and relatively efficient converter of feed to meat. RE 3 did not appear to affect the immune function and response of rabbits.

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