

# Feeding differing direct-fed microbials and its influence on growth and haematological parameters of growing lambs<sup>1</sup>

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**ABSTRACT:** A 4 mo feeding trial was conducted to ascertain the effect of direct-fed microbial (DFM) and their products, namely rumen enhancer three (RE3), *Paenibacillus polymyxa* (P3), and a fermentation product of RE3—RE3 Plus on the growth and haematological profile of lambs at different stages of growth (suckling, weaner, and grower phases). The lambs weighing  $2.5 \pm 2$  kg were blocked by their weights and allotted to four dietary treatments, namely Control, RE3, RE3 Plus, and P3 in a randomized complete block design. Blood sampling of lambs to investigate the effect of the treatments on the haematological and blood biochemistry variables was done on monthly basis and analysis of variance in a repeated measures design was done using the Statistical Analysis System. Feed intake (FI) by lambs was not significantly ( $P > 0.05$ ) influenced by DFM supplementation for all the phases of growth. However, feed conversion ratio (FCR) for the grower

phase of the lamb was higher for T2. Similar ( $P > 0.05$ ) hemoglobin (Hb), red blood cell (RBC), pack cell volume (PCV), mean corpuscular volume, mean corpuscular hemoglobin concentration (MCHC), eosinophil, lymphocyte, and basophil composition were recorded for lambs on the different experimental diets. Neutrophil and monocytes levels were, however, different ( $P < 0.05$ ) for lambs fed the varying dietary treatments with lambs on RE3 Plus diets recording the lowest ( $P < 0.05$ ) neutrophil levels. Monocytes levels were highest ( $P < 0.05$ ) in lambs fed diets fortified with RE3. Sampling period influenced ( $P < 0.05$ ) the Hb, RBC, PCV, MCHC, eosinophil, monocyte, and lymphocyte levels. The trial revealed a significant treatment  $\times$  sampling period interaction ( $P < 0.0001$ ) for the blood parameters examined. The inclusion of the different DFM products had no influence on the growth characteristics and blood profile of growing lambs.

**Keywords:** direct-fed microbial, growth, hematology, lambs

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## INTRODUCTION

The increase in demand for animal products requires strategies that will help to produce substantial quantities of these necessary products within the shortest possible time. Thus, the discovery of the growth-promoting effects of antibiotics on animals was an important milestone

in animal nutrition. Antibiotics, therefore, became very notorious in animal nutrition. The use and misuse of antibiotics in animal feeding has been noted to be one of the main causes of increased occurrence of antibiotic-resistant bacteria (Smith et al., 2002; Hueston et al., 2013). The misuse of these antibiotics has not only been harmful to livestock but humans and other animals through the pollution of the environment as well as the residues that are carried along the food chain (Sarmah, 2006). Furthermore, years of research into several antibiotics and the monies spent on them have gone waste since they are no longer effective in the fight against bacteria (Eliopoulos et al., 2003; Zhang and Yew, 2009). Thus, several legislations have been enacted to curtail the misuse of antibiotics in animal production (Centner, 2008; Maron et al., 2013). Emphases have to be made to the fact that new strategies must be developed if the growing demands for animal products are to be met. Several products, including organic acids, prebiotics, phytogenics, and probiotics or direct-fed microbials (DFM) have been investigated as possible alternatives to in-feed antibiotics.

Probiotics or DFM are preparations or products with defined and viable microorganisms sufficient to alter the intestinal microflora of the host and exert a beneficial health effect (Schrezenmeir and de Vrese, 2001). Darwish et al., (2013) indicated that several animal products in Africa are impregnated with several classes of antibiotics. Thus, if intercontinental trade of animal products were to be pursued, then efforts have to be made to address these issues. There is a dearth of research on the influence of probiotics on different production indices and, moreover, available data show conflicting reports on DFM supplementation; some studies reported significant improvement in animal growth and production (Bonsu et al., 2012; Bonsu et al., 2014), while others reported no influence on the performance (Lee et al., 2010; McAllister et al., 2011). It must also be mentioned that supplementation of DFM or probiotics to animals is a dynamic

part of animal nutrition and new DFM-based products are continually being developed and studied. Some of these are rumen enhance 3 (RE3 Plus), which is a fermentation product of RE3, and P3, which is a *Paenibacillus polymyxa*-based DFM product. The study, therefore, seeks to assess the influence of DFM products (RE3, RE3 Plus, and P3) on the growth and blood constituents of neonatal lambs.

## MATERIALS AND METHODS

The Animal Ethics Committee of Kwame Nkrumah University of Science and Technology approved the protocols used in the trials.

### *Study Duration and Location*

A 4 mo feeding trial was conducted at the Ejura Sheep Breeding Station of the Animal Production Directorate, Ministry of Food and Agriculture (MoFA) in the Ejura-Sekyedumase District of the Ashanti Region of Ghana. Ejura-Sekyedumase is located in the transition zone that is between the semideciduous and Guinea Savanna zones of Ghana.

### *Animals Diets, Treatments, Housing, and Experimental Design*

Twenty-four 4 d old lambs with an average weight of  $2.5 \pm 1.2$  kg were randomly allocated to four treatments (Table 1). All treatments were replicated six times in a randomized complete block design. A repeated measure design was used in the analysis of blood samples. Lambs along with their dams were housed in a cement-block, barn with concrete floors and corrugated aluminum-roofing sheets. Two barns were divided into 24 individual partitions with wood and wire mesh with each partition housing a replicate. Three phases of growth, namely suckling, creep, and grower phases were

**Table 1.** Prewaning and postweaning dietary treatments for preweaning and postweaning phases

Treatment	Prewaning		Postweaning
	Suckling phase [treatment (ml)/10 ml water]	Creep phase [treatment (ml)/kg feed]	
Control	—	—	—
RE3 <sup>1</sup>	1.5	1.5	1.5
RE3 Plus <sup>2</sup>	1.5	1.5	1.5
P3 <sup>3</sup>	1.5	1.5	1.5

DFM products were manufactured by BEST Environmental Technology Inc., Canada.

<sup>1</sup>RE3—Rumen Enhancer contains 99% water, *Lactobacillus* sp., *Bacillus* sp., and *Saccharomyces cerevisiae*.

<sup>2</sup>RE3 Plus is a fermented product of RE3.

<sup>3</sup>DFM product containing *Paenibacillus polymyxa*.

considered in this study. At the suckling phase, the DFM products were added to the water of animals at a rate of 1.5 ml to every 10 ml of water, whilst 1.5 ml DFM was added to every kg of feed in the creep and postweaning phases.

### Feeding

The composition of diets prepared for the lambs at the preweaning and postweaning phases are shown in Table 2. The composition of the diets was typical of diets fed to lambs in the Ejura Sheep Breeding Station. No diet was prepared for lambs in the suckling phase. A creep partition was created for lambs in the creep phase to prevent dams from getting access to the creep feed. Diets were compounded every 3 d and the DFM products were added. Lambs were offered a 16.1% and an 18.6% crude protein diet in the creep and postweaning phase, respectively.

### Parameters Measured

The quantities of feed offered and refused were recorded daily and the differences were considered as the feed intake. Weight gain was recorded fortnightly and served as basis for calculating the growth rate of the lambs. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to weight gain. Blood samples were collected via the jugular vein from lambs monthly for haematological assays.

### Statistical Analysis

The PROC MIXED procedure of Statistical Analytical System (SAS Institute Inc., 2006) was used to analyze all the experimental data. Where

**Table 2.** Composition of preweaning and postweaning diets (as fed basis)<sup>1</sup>

Ingredients	Creep phase	Postweaning phase
	Level of incorporation	Level of incorporation
Wheat bran	38	40
Maize	30	10
Concentrate	9	7
Cotton seed meal	20	40
Dicalcium phosphate	1	1
Salt	1	1
Oyster shell	1	1
%CP in diet	16.1	18.6

CP, crude protein.

<sup>1</sup>Samples were collected from the mill, homogenized, and then sub-sampled for analysis.

there was significant effect at ( $P < 0.05$ ) treatment, means were compared by least square means. The mean separation was tested by Waller Duncan Multiple Range test in SAS.

## RESULTS AND DISCUSSION

Results of the intake and growth performance indicated a nonsignificant ( $P > 0.05$ ) influence of the treatment on dry matter intake and weight gain by the lambs in all the phases of growth (Table 3). Similarly, treatment effect was not significant ( $P > 0.05$ ) on the growth performance of lambs from suckling phase to the grower phase. The results contrast the outcome of study by Adams et al. (2008), where DFM was administered to young calves. An improvement in weight gains were recorded during preweaning and postweaning phases in the study. Adeshinwa et al. (2016), however, reported that addition of DFM products to the diets of livestock did not elicit any influence on the growth of livestock, which is in consonance with the data reported in this study. Zulkifli et al. (2000) had earlier indicated that the high temperatures that are characteristic of the tropics are probably responsible for the inconsistency in data on the response of livestock to probiotics.

Likewise, the FCR was similar among the treatments for lambs in the creep phase. The FCR for sheep on control- and P3-based diets were better ( $P = 0.023$ ) than sheep on diets fortified with RE3 in the grower phase; however, sheep fed RE3 Plus-containing diets had FCRs, which were similar ( $P > 0.05$ ) to sheep on all the other experimental diets. The authors Zulkifli et al. (2000) further indicated that adding DFM or additives that may boost feed intake may improve intake in livestock even in areas with temperatures that may be stressful to livestock. Thus, feeding such additives to livestock in such areas may result in reduced efficiency since the animals may eventually dissipate the energy obtained from the feed. This may therefore explain in part why DFM-fortified diets resulted in poorer efficiencies than the Control diet at the grower phase of this study.

### Blood Profile

A significant treatment  $\times$  sampling period interaction ( $P < 0.0001$ ) was recorded for the erythrocytes and leucocytes examined (Tables 4 and 5). The treatments did not influence ( $P > 0.05$ ) the red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV),

**Table 3.** Growth performance of sheep from suckling to grower phase

TRT	Control	RE3	RE3 Plus	P3	P-value
Suckling phase					
ADI, kg	—	—	—	—	—
INIT, kg	2.78	3.3	3.64	3.37	0.520
FWT, kg	5.23	6.08	6.18	6.37	0.070
WG, kg	2.45	2.78	2.54	3.00	0.910
ADG, kg	0.09	0.10	0.09	0.11	0.910
FCR	—	—	—	—	—
Creep phase					
ADI, kg	0.11	0.16	0.13	0.15	0.443
INIT, kg	5.40	6.30	6.22	6.38	0.514
FWT, kg	9.37	11.13	10.30	11.31	0.599
WG, kg	3.97	4.83	4.08	4.93	0.590
ADG, kg	0.071	0.086	0.073	0.088	0.590
FCR	1.48	1.79	1.67	1.91	0.733
Grower phase					
ADI, kg	0.34	0.49	0.37	0.44	0.262
INIT, kg	9.58	12.47	11.04	12.48	0.342
FWT, kg	11.65	14.10	12.70	14.53	0.240
WG, kg	2.07	1.63	1.66	2.55	0.356
ADG, kg	0.074	0.058	0.059	0.091	0.356
FCR	4.66b	9.11a	6.69ab	5.076b	0.023
Overall (creep-grower phase)					
ADI, kg	0.51	0.76	0.58	0.69	0.285
INIT, kg	2.78	3.30	3.64	3.37	0.013
FWT, kg	11.65	14.10	12.7	14.53	0.345
WG, kg	8.87	10.80	9.06	11.17	0.443
ADG, kg	0.079	0.097	0.081	0.10	0.449
FCR	6.33	7.74	7.14	7.32	0.527

TRT, treatment; ADI, average daily intake; INIT, initial body weight; FWT, final body weight; WG, weight gain; ADG, average daily weight gain.

mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) of lambs, eosinophils, lymphocytes, and basophils. This is in consonance with a study by [Hossein-Ali et al. \(2014\)](#) who reported no significant influence on white blood cells (WBCs) when probiotics-based diet was fed to lambs. However, the erythrocytes response to DFM treatment contrasts the results obtained by the same authors who recorded a significant decline in the erythrocytes values in their studies. [Park and Kim \(2015\)](#) also recorded no differences in WBC, RBC, and lymphocytes when broilers were fed diets containing *Bacillus subtilis*-based DFM.

The generally low RBC levels may be a sign of anemia in all the sheep. Also, the low RBC levels may be peculiar to sheep breeds within the region. In a study by [Baiden et al. \(2007\)](#) on the influence of feeding differing cassava pulp-based diets to ruminant livestock in Ghana, similar RBC values

were recorded for sheep. Earlier ([Ghorbani et al., 2002](#)), the addition of *Propionibacterium* to rations of feedlot cattle resulted in no differences in their PCV. Also, [Sarker et al. \(2010\)](#) had opined that the inclusion of fermented green tea probiotic to rations for calves had no influence ( $P > 0.05$ ) on the RBC, MCH, and MCHC composition of their blood but further reported significant reductions ( $P < 0.05$ ) in their Hb and MCV. In another study involving growing pigs, [Chen et al. \(2005\)](#) indicated that *Bacillus* supplementation resulted in no changes in erythrocytes as reported in this study. Again the addition of differing DFM products had no effect on the MCHC of pigs at different stages of growth ([Petrovič et al., 2009](#); [Kunavue and Lien, 2012](#)). On the contrary, the higher MCH and MCHC levels recorded in this study when compared with the normal physiological ranges is in consonance with a study by [Siadati et al. \(2017\)](#) who reported high MCHC in Japanese quails following probiotic supplementation.

Comparison of the WBCs concentration measured showed no significant difference ( $P > 0.05$ ) for all white cells except for neutrophils and monocytes, which was significantly higher ( $P < 0.05$ ) in lambs fed P3 containing diets and in lambs on diets containing RE3, respectively. This is in agreement with a study by [Rahman et al. \(2007\)](#), as cited in [Dabiri et al \(2016\)](#), who reported no significant effect on the WBCs when suckling lambs were fed creep feed containing yeast. It is established that probiotics increase macrophage activity that stimulates the immune system, which act by increasing the phagocytic ability of microorganisms, hence the increased neutrophils levels in response to DFM treatment. The neutrophils values were similar to those recorded for lambs on diets fortified with RE3 and the Control. Lambs fed the RE3 Plus-containing diets recorded the least ( $P < 0.05$ ) neutrophil values. It must be stated that neutrophil values were similar ( $P > 0.05$ ) for lambs fed the RE3- and RE3 Plus-fortified diets. Monocyte levels were highest ( $P < 0.05$ ) in the in lambs on diets containing RE3 but similar for those on the remaining treatments.

According to [Prieto et al. \(2014\)](#), higher levels of granulocytes, such as those for eosinophils reported in this study could be attributed to immune response, which is characteristic of the inflammatory response in the guts of livestock fed these DFMs.

Sampling period (SP) significantly influenced ( $P < 0.05$ ) the Hb, RBC, PCV, MCHC, eosinophils, monocytes, and lymphocytes in lambs but had no effect ( $P > 0.05$ ) on the levels of neutrophils and

**Table 4.** Effect of direct-fed microbial on erythrocytes

Treatment	SP, month	Erythrocytes					
		Hb, g/dl	RBC, 10 <sup>6</sup> /μL	PCV, %	MCV, μm <sup>3</sup>	MCH, pg	MCHC, %
Reference range <sup>1</sup>		9–15	9–15	27–45	28–40	8–12	31–34
Control		10.05	7.60	29.21	39.30	13.57	34.84
RE3		10.64	7.95	30.00	37.71	13.61	36.35
RE3 Plus		10.63	8.13	29.95	36.18	13.06	36.12
P3		10.56	7.83	29.65	37.53	13.25	33.94
SE		0.69	0.31	1.48	1.96	0.75	1.40
	1	11.49a	8.14b	30.55b	37.47	14.20	36.77a
	2	11.20a	8.93a	33.60a	37.85	12.68	33.40b
	3	7.86b	5.71c	20.67c	37.54	13.93	37.67a
	4	11.33a	8.72a	34.00a	37.85	12.68	33.40b
SE		0.66	0.31	1.50	2.03	0.73	1.38
Statistical interaction							
Control*sp		****	****	****	****	****	****
RE3*sp		****	****	****	****	****	****
RE3 Plus*sp		****	****	****	****	****	****
P3*sp		****	****	****	****	****	****

Means with the common letter within treatments and sampling periods (SP) are not significantly different based on comparison of least squares means within PROC MIXED of SAS. \*\*\*\* $P < 0.0001$ .

<sup>1</sup>Source: Aiello (2000).

**Table 5.** Effect of direct-fed microbial on leucocytes

Treatment	SP, month	Leucocytes				
		NEUT, %	EOSIN, %	MONO, %	LYMPH, %	BASO, %
Reference range <sup>1</sup>		10–50	0–10	0–6	40–75	0–3
Control		46.94a	11.83	5.22b	35.89	0.11
RE3		45.69ab	12.24	6.90a	34.46	0.25
RE3 Plus		43.80b	14.00	5.64b	36.10	0.13
P3		47.07a	11.94	5.37b	35.24	0.07
SE		1.46	1.74	0.65	1.63	0.14
	1	46.50	9.78b	5.40b	37.94a	0.11
	2	44.74	17.35a	6.83a	30.65b	0.15
	3	46.40	10.34b	5.12b	37.68a	0.15
SE		1.27	1.49	0.53	1.39	0.12
Statistical interaction						
Control*sp		****	****	****	****	****
RE3*sp		****	****	****	****	****
RE3 Plus*sp		****	****	****	****	****
P3*sp		****	****	****	****	****

Means with the common letter within treatment and sampling periods (SP) are not significantly different based on comparison of least squares means within PROC MIXED of SAS. \*\*\*\* $P < 0.0001$ .

NEUT, neutrophils; EOSIN, eosinophils; MONO, monocytes; LYMPH, lymphocytes; BASO, basophils.

<sup>1</sup>Source: Aiello (2000).

basophils. The Hb content of blood for lambs was lowest ( $P < 0.05$ ) on the third month (SP 3) but months 1, 2, and 4 were similar. When compared with the normal Hb range for sheep (Table 4), it was realized that all values were in range with the exception of the values recorded for SP 3. The RBC and PCV content were lower in the first month for all lambs but significantly ( $P < 0.05$ ) higher than levels

recorded for the third month. The average RBC levels of lambs on different treatments and averages of all the sampling periods were lower than normal ranges of (9–15 μl) recorded for sheep (Aiello, 2000). This may be indicative of anemia during the study period, especially the third month of sampling, but the relatively higher values of PCV, MCV, and MCH above the normal range could have suppressed its

effect (Hossein-Ali et al., 2014). The mean corpuscular hemoglobin concentration was, however, higher ( $P < 0.05$ ) for lambs on the first and third months of sampling compared with the second and fourth months of sampling. The MCHC though within normal ranges, the dip during the second and fourth months of study may possibly be due to temporal anemia in the lambs receiving the DFM.

All the figures obtained for neutrophils and basophils were normal when compared with the ranges for normal physiological activities in sheep (Table 5). On the other hand, the lymphocyte levels were generally lower than normal physiological values. Eosinophil and monocyte levels recorded for SP 2 were significantly ( $P < 0.05$ ) higher than those recorded for SP 1 and SP 3. A reverse trend was, however, recorded with regards to lymphocytes where lambs recorded significantly ( $P < 0.05$ ) higher levels in SP 1 and SP 3 compared with SP 2. Only eosinophil levels for the first sampling period were within the normal ranges reported by Aiello (2000) but sheep had higher values for the remaining sampling period. This may be attributable to the response of eosinophilia to possible presence of helminth load during that period (Olayemi et al., 2015). The second month of sampling, which coincides with the dry season, witnessed the highest-level monocytes in the blood. The relative monocytosis, according to the same authors, may be ascribed to chronic respiratory infection, which is prevalent during the dry season when the study was carried out.

It must be stated that the addition of DFMs or probiotics have yielded inconsistent results on the blood profile of livestock. These findings, according to Alkhalif et al. (2010), could be attributed to the differences in the type and number of microbes that may be used in the probiotic product. Al-Dohail et al. (2009), on the other hand, attributed some of these differences to environmental factors as well as the differences in species of livestock used in these studies.

In conclusion, the study demonstrated that DFM supplementation has no influence on the growth and blood profile of growing sheep. The study demonstrated the need for varying or increasing the levels of the probiotics to ascertain the optimum inclusion levels that would further improve animal performance.

*Conflict of interest statement.* None as stated.

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