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# Effect of Multi-enzymes Supplementation in Egg type Chicks Diet with Varying Protein and Energy levels During Hot-dry Summer

### S. P. Dukare, A. B. Mandal, J. J. Rokade and O. P. Dinani<sup>\*</sup>

ICAR-Central Avian Research Institute, Izatnagar, Uttar Pradesh (243 122), India

Corresponding Author	Article History
O. P. Dinani	Article ID: AR1837
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### Abstract

An experiment was conducted to evaluate the effects of dietary inclusion of (multi-enzymes) maxi-zyme at different energy and protein level on performance of egg-type chicks during hot-dry summer. After feeding a standard diet from 0-21d of age, the chicks were randomly distributed into four dietary treatment groups *viz.*,  $T_1$  with high energy 2800 kcal ME kg<sup>-1</sup> and high protein 18.0% (HEHP), T2 HEHP diet with maxi-zyme 0.025% and  $T_3$  with low energy 2700 kcal ME kg<sup>-1</sup> low protein 17.34% (LELP) and T4 with LELP diet with maxi-zyme 0.025%. Each treatment is with fifty birds divided in five replicated of ten birds each. Experiment was carried out during hot-dry summer (April-May, 28.0±0.12 °C to 35.25±0.37 °C and RH %: 68.95±0.90 to 79.15±0.61). Production performance, immune organ weight, blood biochemical and intestinal morphometry were measured at 42<sup>nd</sup> and 63<sup>rd</sup> day post hatch. Body weight gain, Feed intake and FCR improved significantly (P<0.05) in HEHP+maxi-zyme group. H:L ratio significantly (*p*<0.05) reduced in maxi-zyme supplemented group at 42<sup>nd</sup> as well as 63<sup>rd</sup> day of age. Immune organ like thymus, spleen and bursa had significantly (*p*<0.05) higher weight in maxi-zyme supplemented group. Total protein (*p*<0.001), SGOT (*p*<0.001), SGPT (*p*<0.001), creatinine (*p*<0.05) were significantly improved while cholesterol, uric acid & ALP was significantly (*p*<0.001) reduced in maxi-zyme supplemented diets. Villus height & crypt depth improved significantly (*p*<0.001) in maxizyme supplemented group at 42<sup>nd</sup> as well as 63<sup>rd</sup> day of age. Thus, it was concluded that high-energy (2800 kcal ME kg<sup>-1</sup>) and high protein (18% CP) diet was beneficial for egg type starting chicks during dry summer. Moreover, the inclusion of maxi-zyme @ 0.025% gave further advantage to improve performance and reduce thermal stress.

Keywords: Egg-type chicks, heat stress, maxi-zyme and nutritional plane

### 1. Introduction

There was continuous research is going on to improve nutritive value of feed by enzyme supplementation. In addition to the use of the conventional xylanase, glucanase, phytase, and, more recently, multi-carbohydrase preparations, the application of normal digestive tract enzymes (i.e., protease, amylase, lipase) have also been proposed (Jiang et al., 2008; Angel et al., 2011; Kalmendal and Tauson, 2012; Yegani and Korver, 2013). The application of these enzymes has been driven by the idea that newly hatched chicks may be deficient in key digestive enzymes; this has been corroborated by Nitsan et al. (1991); Noy and Sklan (1995); Jin et al. (1998), who showed that specific activities of lipase, amylase, and trypsin rapidly increase up to 2 to 3 wk post hatch. Consequently, it has been suggested that the immaturity of the digestive system of young chicks may result in the poor utilization of dietary nutrients (Jin et al., 1998), and nutrient digestion rather than the ability to absorb nutrients has been indicated

to be the primary limiting factor (Parsons, 2004). The use of exogenous enzymes in the diets of broilers is an important tool to reduce these costs. Even with highly digestible diets, such as those based on corn and soybean meal, the addition of enzymes may improve the dietary energy, and the gut viscosity may be decreased (Hahn-Didde and Purdum, 2014). However, according to (Choct, 2006), the benefits of including enzymes in broiler chicken nutrition include not only better performance but also environmental stress. Furthermore, the enzymes could improve the digestion and absorption of the majority of the nutrients prior to the establishment on environment which is favorable to bacterial growth (Bedford, 2000). Enzymes enhance nutrient delivery to the host and provide substrate (fermentable oligosaccharides) for the microbiota (Cowieson, 2012), consequently improving the intestinal health. Different blends of enzymes, with specificity to different substrates, may have beneficial effects in promoting the intestinal health of birds that are raised in poor environmental conditions.

## 2. Materials and Methods

A feeding trial was conducted during 0 to 63 days of age in layer starting chicks to study the efficacy of maxi-zyme on performance during hot-dry summer (April-May, 28.0±0.12 °C to 35.25±0.37 °C and RH, % 68.95±0.90 to 79.15±0.61) involving day-old CARI Sonali layer chicks (n=200). After feeding standard diet for 0 to 21d of age, the chicks were randomly distributed into four dietary treatment groups *viz.*,  $T_1$  (high energy high protein-HEHP),  $T_2$  (HEHP diet with maxi-zyme @ 0.025%).  $T_3$  (Low energy low protein-LELP) and T4 (LELP diet with maxi-zyme @ 0.025) each with fifty birds divided in five replicates of ten birds each up to 63 days of age. MOS was incorporated both in starter HEHP (18% CP and 2800 kcal ME kg<sup>-1</sup>) and LELP (17.34% CP and 2700 kcal ME kg<sup>-1</sup>) diets at two levels (0 or 0.025%). The ingredient and chemical composition of diets are presented in Table 1.

Data regarding growth performance in the control and experimental group were recorded every week from 21-63rd days of age. Lymphoid organ weights (bursa of Fabricius, spleen, and thymus) were recorded on the 42<sup>nd</sup> and 63<sup>ed</sup> day expressed as percent (relative yield) of live weight. Blood samples (1 ml) were collected from the wing vein using 24 gauge needles in  $K_3$ -EDTA tubes on  $42^{nd}$  and  $63^{rd}$  day of age. Hemoglobin concentration (g dl<sup>-1</sup>) in the whole blood was estimated by cyano-methaemoglobin method. Blood smears prepared from fresh blood smear were stained by Giemsa stain (1:9 Dilution for 45 min) to calculate Heterophil to lymphocyte (H:L) ratio. Serum samples were separated after blood collection and subjected to different blood biochemical tests like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total protein and total cholesterol using standard KIT. On 42<sup>nd</sup> and 63<sup>rd</sup> day after slaughter jejunum samples were collected and histomorphology of villas height depth and the ratio was observed. The data were analyzed following 2×2 factorial designs (Snedechor and Cochran, 1989).

# 3. Results and Discussion

Weight gain differs significantly (p<0.001) due to the incorporation of maxi-zyme in the diet in all the growth phases and the plane of nutrition during 43-63d of age. Reduced BWG has been reported in birds fed diets with low (Reece et al., 1984) and excessive (Cornejo et al., 1991) energy. It is thus beneficial for birds to consume diets with proper energy levels to optimize performance. Zanella et al. (1999) found that supplementation of Avizyme to corn soybean based diet had improved the body weight gain. Cowieson and coworkers (2006) reported a similar result in which higher BWG was observed in birds fed normal nutrients with enzyme supplementation. This might be a result of an incidental optimal dietary ratio of protein (or amino acid) to energy which stimulated enhanced FI and BWG (Bartov and Plavnik, 1998), while enzyme supplementation may have

Ingredients (kg 100 kg <sup>-1</sup> )	HEHP	LELP
Yellow maize	55.8	50.7
DORB	12.935	21.475
Soybean meal	18.7	15.3
Rapeseed meal	5	5
Roasted guar	5	5
Limestone powder	1	1.1
Dicalcium phosphate	0.95	0.75
Common salt	0.3	0.3
Lysine	0	0.06
TM premix 1	0.1	0.1
Vit premix 2	0.15	0.15
B comp	0.015	0.015
Ch Chloride	0.05	0.05
Total	100	100.0
Nutrient composition (As fed	basis)	
ME, kcal/kg***	2800.41	2700.32
Crude Protein, %**	17.99	17.33
Lysine, % <sup>***</sup>	0.85	0.85
Methionine, %***	0.33	0.33
Threonine	0.83	0.77
Calcium, %**	1.02	1.01
Total Phosphorus, %**	0.75	0.75
Available Phosphorus, %***	0.45	0.45

Trace mineral premix includes 0.1, vitamin premixes 0.15. Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30 and Cu- 4 mg kg<sup>-1</sup> diet. The vitamin premixes supplied vitamin A 8250 IU, vitamin D<sub>3</sub> 1200 ICU; vitamin K 1 mg; vitamin E 40 IU, vitamin B<sub>1</sub> 2 mg, vitamin B<sub>2</sub> 4 mg, vitamin B<sub>12</sub> 10 mcg; niacin 60 mg; pantothenic acid 10 mg and choline chloride 500 mg kg<sup>-1</sup> diet; Representative samples of practical feed ingredients and test diets used in the study were analyzed. (AOAC, 1990).

released additional energy and altered the ratio which limited FI in birds.

Feed intake differs significantly (*p*<0.001) due to the incorporation of maxi-zyme in the diet in all the growth phases and the plane of nutrition during 43-63d of age, while interaction was not significant at any growth phase. Kocher et al. (2002) reported that addition of multi-enzymes to cornsoybean based diet showed a decrease in the feed intake. The significantly improved feed conversion ratio in the presence of the multi-enzyme may be a consequence of decreasing microbial colonization in the gut, thereby improving the

### availability of nutrients.

The feed conversion ratio (FCR) differed significantly (*p*<0.001) due to maxi-zyme as well as the plane of nutrition in all the phases (22 to 42, 43 to 63 or 22 to 63d of age) but the interaction of these two major effects did not influence FCR (Table 2). The birds with maxi-zyme fed diet had lower feed intake in all the growth phases. Zanella et al. (1999) observed that addition of Avizyme to corn-soybean based broiler diet improved the feed efficiency. Enzyme supplementation might improve broiler performance by improving nutrient digestibility. This mechanism might be induced, at least partially, by a reduction of the viscosity Lazaro et al. (2003). The relative higher protein retention may also have contributed to a better FCR due to reduced loss of endogenous

## secretion (Cowieson et al., 2003)

Though body weight gain did not differ due to the dietary plane but the values were apparently higher in HEHP diet. In contrary, body weight increased by increasing protein level from 18% to 20% CP (Toppo et al., 2004). In the present study increment of protein was much less and the ratio of energy to protein remained similar, which might be the possible reasons for a similar gain in body weight. Feed intake increased in low energy low protein diet, which may be attributed to dietary energy concentration. The feed intake increased significantly with low energy diet (2600 vs. 2800 kcal ME kg<sup>-1</sup>) but there was no change in total feed intake by increasing crude protein levels from 18 to 22% at any energy level (Toppo et al., 2004). Feed conversion ratio was significantly (p<0.05) poor in low

Table 2: Body weight gain, feed intake and feed conversion ratio in different treatments										
EP	Maxi-	BWG	BWG	BWG	FI	FI	FI	FCR	FCR	FCR
	zyme	(3-6 wk)	(6-9 wk)	(3-9 wk)	(3-6 wk)	(6-9wk)	(3-9 wk)	(3-6 wk)	(6-9 wk)	(3-9 wk)
HEHP	0	230.2	360.3	589.5	772	1283	2040	3.35	3.26	3.46
HEHP	0.025	246.3	402.5	650.3	736	1119	1816	2.98	2.78	2.79
LELP	0	220.8	365.9	598.0	815	1405	2206	3.69	3.83	3.68
LELP	0.025	245.6	418.7	619.9	742	1213	1845	3.02	2.89	2.97
SEM	2.85	7.82	9.83	7.18	31.37	33.29	0.05	0.05	0.02	
HEHP		230.2b	381.4b	619.9b	754b	1201b	1928b	3.17b	3.17b	3.12b
LELP		233.2a	392.3a	608.9a	778a	1309a	2025a	3.35a	3.36a	3.33a
Maxi-zyme	0	225.5x	363.2×	593.7×	793 <sup>×</sup>	1344×	2123 <sup>×</sup>	3.52×	3.70 <sup>×</sup>	3.57×
Maxi-zyme	0.025	245.9 <sup>y</sup>	410.6 <sup>y</sup>	635.1 <sup>y</sup>	739 <sup>y</sup>	1166 <sup>y</sup>	1830 <sup>y</sup>	3.00 <sup>y</sup>	2.83 <sup>y</sup>	2.88 <sup>y</sup>
Probability										
EP		<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03	<i>p</i> <0.03
Maxi-zyme		<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.01	<i>p</i> <0.001	<i>p</i> <0.001
EP X Maxi-zyme		NS	NS	NS	NS	NS	NS	NS	NS	NS

abxyValues bearing different superscript differed significantly (p<0.01); NS, Non significant

energy low protein diet as in earlier experiment FCR remained poor than in high energy level in CARI Sonali chicks, and feed conversion ratio tended to be higher at low protein (18%) diet (Toppo et al., 2004).

Supplementation of maxi-zyme caused significant improvement in relative weights of spleen, thymus, and bursa during 43 to 63 d post-hatch during hot humid climate (Table 3). There was no interaction and different energy protein level effect. In the present study, significantly (p<0.001) lower H:L ratio in maxi-zyme fed bird as compared to control birds in HEHP and LELP feed during 42<sup>nd</sup> and 63<sup>rd</sup> d of age (Table 4 and Table 5).

The serum total protein was significantly (p<0.001) higher in maxi-zyme supplemented birds as compared to control in both HEHP and LELP birds at 43 to 63 d of age during hot dry summer (Table 4 and 5). Maxi-zyme supplementation significantly

(p<0.001) reduced the serum cholesterol concentration during 43 to 63 d of age as compared to unsupplemented birds in both HEHP and LELP during hot-dry summer. During 43 to 63 d of age aspartame aminotransferase (AST) and alanine aminotransferase (ALT) levels increased significantly (p<0.001) with higher concentration in maxi-zyme fed birds as compared to control birds in both HEHP and LELP feed. Maxi-zyme supplementation significantly (p<0.001) reduced the serum Alkaline Phosphates concentration during 43 to 63d of age as compared to control birds in both HEHP and LELP during hot summer. Maxi-zyme supplementation significantly (p<0.001) reduced the serum uric acid concentration at 42<sup>nd</sup> and 63<sup>rd</sup> day of age as compared to control birds in both HEHP and LELP during hot-dry summer. During 43 to 63d of age, creatinine levels increased significantly (p<0.001) with supplementation of maxi-zyme fed birds as compared to control birds in both HEHP and LELP. During 42<sup>nd</sup> and 63<sup>rd</sup> d of age, villus height

Table 3: Yield of immune organs (Percent of body weight) in different treatments									
EP	Maxi-zyme		6 week		9 week				
	(%)	Thymus	Bursa	Spleen	Thymus	Bursa	Spleen		
НЕНР	0	0.292	0.120	0.216	0.529	0.112	0.202		
	0.025	0.367	0.181	0.294	0.483	0.195	0.298		
LELP	0	0.291	0.128	0.211	0.505	0.119	0.216		
	0.025	0.373	0.143	0.274	0.443	0.189	0.289		
SEm±	0.01	0.01	0.01	0.02	0.01	0.01			
НЕНР		0.329	0.150	0.255	0.506	0.153	0.250		
LELP		0.332	0.135	0.242	0.474	0.154	0.252		
Maxi-zyme	0	0.291×	0.124 <sup>×</sup>	0.213 <sup>x</sup>	0.517×	0.115 <sup>×</sup>	0.209×		
Maxi-zyme	0.025	0.370y	0.162 <sup>y</sup>	0.284 <sup>y</sup>	0.463 <sup>y</sup>	0.192 <sup>y</sup>	0.293 <sup>y</sup>		
Probability									
EP		NS	NS	NS	NS	NS	NS		
Maxi-zyme		<i>p</i> >0.001	<i>p</i> >0.001	<i>p</i> >0.001	<i>p</i> >0.003	<i>p</i> >0.003	<i>p</i> >0.000		
EP X Maxi-zyme		NS	NS	NS	NS	NS	NS		

abxyValues bearing different superscript differed significantly (*p*<0.01); NS: Non significant

Table 4: Blood biochemical parameter and H:L ratio at 6 weeks of age in different treatments									
EP	Maxi-	Protein	SGOT	SGPT	Choles-	Uric acid	Alkaline	Cre-	H:L ratio
	zyme				terol		Р	atine	
HEHP	0	3.14	84.44	2.81	171.56	5.47	46.70	0.69	0.47
	0.025	4.28	108.25	3.72	136.32	3.78	33.53	0.78	0.38
LELP	0	3.08	91.37	2.90	182.65	5.61	48.27	0.71	0.45
	0.025	4.13	106.26	3.69	153.71	3.91	35.82	0.80	0.37
Sem	0.04	0.61	0.07	0.81	0.07	0.74	0.01	0.01	
HEHP		3.71a	96.34	3.26	153.94	4.62	40.11	0.73	0.42
LELP		3.60b	98.81	3.29	168.18	4.76	42.04	0.75	0.41
Maxi-zyme	0	3.11 <sup>×</sup>	87.90 <sup>×</sup>	2.85×	177.10 <sup>×</sup>	5.54×	47.48 <sup>×</sup>	0.70 <sup>×</sup>	0.46 <sup>×</sup>
Maxi-zyme	0.025	4.20y	107.25y	3.70y	145.01 <sup>y</sup>	3.84 <sup>y</sup>	34.67 <sup>y</sup>	0.79 <sup>y</sup>	0.37 <sup>y</sup>
Probability									
EP		NS	NS	NS	NS	NS	NS	NS	NS
Maxi-zyme		<i>p</i> >0.000	<i>p</i> >0.000	<i>p</i> >0.000	<i>p</i> >0.000	<i>p</i> >0.000	<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.003
EP X Maxi-zyme		NS	NS	NS	NS	NS	NS	NS	NS

abxyValues bearing different superscript differed significantly (p<0.01); NS: Non significant

Table 5: Blood biochemical parameter and H:L ratio at 9 weeks of age in different treatments									
EP	Maxi-zyme	Protein	SGOT	SGPT	Cholesterol	Uric acid	Alkaline P	Creatine	H:L ratio
НЕНР	0	3.80	72.67	2.82	181.73	5.71	54.87	0.78	0.41
	0.025	5.47	108.22	3.47	135.47	3.62	36.72	0.72	0.34
LELP	0	3.74	71.71	2.89	183.29	5.74	56.81	0.76	0.42
	0.025	5.63	105.87	3.27	138.78	3.37	38.55	0.70	0.33
SEm±		0.08	0.98	0.09	1.51	0.08	0.79	0.01	0.01

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EP	Maxi-zyme	Protein	SGOT	SGPT	Cholesterol	Uric acid	Alkaline P	Creatine	H:L ratio
HEHP		4.63	90.44	3.14	158.60	4.66	45.79	0.75	0.37
LELP		4.68	88.79	3.08	161.03	4.55	47.68	0.73	0.37
Maxi-zyme	0	3.77×	72.19 <sup>×</sup>	2.85×	182.51×	5.72×	55.84 <sup>×</sup>	0.77×	0.41 <sup>×</sup>
Maxi-zyme	0.025	5.55y	107.04 <sup>y</sup>	3.37 <sup>y</sup>	137.12 <sup>y</sup>	3.49 <sup>y</sup>	37.63 <sup>y</sup>	0.71 <sup>y</sup>	0.33 <sup>y</sup>
Probability									
EP		NS	NS	NS	NS	NS	NS	NS	NS
Maxi-zyme		<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.00	<i>p</i> >0.04	<i>p</i> >0.01
EP×Maxi-zyme		NS	NS	NS	NS	NS	NS	NS	NS

abxy Values bearing different superscript differed significantly (p<0.01); NS, Non significant

increased significantly (p<0.001) with a higher value in maxizyme fed birds as compared to control birds in both HEHP and LELP feed. Maxi-zyme supplementation significantly (p<0.001) reduced the crypt depth during 42<sup>nd</sup> and 63<sup>rd</sup> d of age as compared to control birds in both HEHP and LELP feed during hot-dry summer.

# 4. Conclusion

High-energy (2800 kcal ME kg<sup>1</sup>) and high protein (18% CP) diet was beneficial for egg type starting chicks during dry summer. Moreover, the inclusion of maxi-zyme @ 0.2% gave further advantage to improve performance and reduce thermal stress as evidenced through zoo-technical indices and blood biochemicals.

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