ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 10 (10): 786-795, 2011
ISSN 1682-8356

Evaluation of Rovabio Max™ in Normal and Reduced-Nutrient Corn-Soybean Meal and Distillers Dried Grains with Solubles Diets for Broilers[1]

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Abstract: A study was conducted to evaluate the effects of adding Rovabio Max™, an exogenous enzyme containing xylanase, β-glucanase, pectinase, mannanase, phytase and α-galactosidase activity to broiler diets with normal or reduced nutrient levels. Positive control diets for 0-3 wk and 3-6 wk were prepared based on NRC (1994) recommendations with Lys adjusted to 1.2% for 0-3 wk and 1.1% for 3-6 wk. These diets were formulated with or without the addition of 20% Distillers Dried Grains with Solubles (DDGS) of known composition. Reduced nutrient diets were prepared by reducing dietary metabolizable energy by 40 kcal/lb (88 kcal/kg), Ca by 0.10% and available P by 0.12% (EPC) and by an additional reduction of 5% in essential amino acids (EPC+AA). All diets were then fed with or without the addition of Rovabio Max™ at 200 g/ton as suggested by the manufacturer. Each diet was fed to four pens of 60 male birds of a commercial strain (Cobb 500) maintained in litter floor pens. Birds were weighed and feed consumption determined at 21 and 42 d of age. Two birds per pen were killed and tibia ash determined at 21 and 42 d; five birds per pen were killed at 42 d to determine carcass dressing percentage and parts yield. Birds fed the EPC diets did not differ significantly from those fed the positive control diet for body weight, mortality, tibia ash, dressing percentage, or breast meat yield at any point in the study. Additional reduction in amino acid content of the diets (EPC+AA) resulted in a significant decrease in 42 d body weight and a reduction in 21 d tibia ash compared to birds fed the positive control diet. Numerical differences in feed conversion were observed for both levels of nutrient reduction but not commensurate to the reduction in dietary energy content. Therefore, response to any exogenous enzyme might be expected to be minimal. The only positive response to the addition of Rovabio Max was a significant improvement in 21 d tibia ash, due to the phytase activity of the enzyme combination. Inclusion of 20% DDGS in diets in the present study had no adverse effects on broiler performance. The response to the DDGS was significantly higher in diets with reduced amino acid levels; the DDGS sample in the present study contained similar levels of crude protein as in the assigned nutrient matrix but the quality of the product appeared to be superior as judged by the light color indicating minimal overheating. It would appear that the level of nutrient reduction in the present study was not sufficient to result in significant reduction in performance, other than tibia ash at 21 d. In order to demonstrate positive effects from this or other enzymes, it is necessary to have diets with a greater degree of nutrient reduction than imposed in the present study.

Key words: Rovabio Max™, soybean meal, poultry industry

INTRODUCTION

Soybean meal is widely recognized as a high-quality protein source and is the leading protein supplement in the United States, Brazil and many other major poultry producing countries. However, soybean meal also contains approximately 30% carbohydrate and the digestibility of this fraction is considered very poor. Honig and Rakis (1979) noted that the carbohydrate fraction is made up almost equally of various oligosaccharides and polysaccharides. The poor digestibility of the oligosaccharide fraction leads to covert losses to the poultry industry, with one of the most obvious being the loss of potential energy. Studies on metabolizable energy indicate that soybean meal and dehulled soybean meal contain about 5-6% more gross energy than corn but 54 and 42% less metabolizable energy (Hill *et al*., 1960; Hill and Renner, 1960; Potter and Matterson, 1960; Sibbald and Slinger, 1962), indicating that some component(s) of the soybean meals are poorly digested and metabolized.

Increasing production of ethanol from corn has resulted in large quantities of the residue of grain fermentation, known as Distillers Dried Grains with Solubles (DDGS). The DDGS contain much lower quantities of starch and higher levels of fiber than the original grain. Consequently, the usable energy content of DDGS is much lower than that of the initial grain. Min *et al*. (2009) reported that broiler chicks digested 76.4% of the gross energy in a typical corn-soybean meal diet but only 67.6% of the gross energy in a diet with 30% DDGS.

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Many enzymes have been found to be beneficial when added to poultry diets containing carbohydrates or protein sources containing high level of Non-Starch Polysaccharides (NSP), such as wheat, barley, rye, peas and lupins (Bedford and Morgan, 1996; Chesson, 2001; Acamovic, 2001). Few studies, however, have consistently demonstrated a response to such enzymes when the diets are composed primarily of corn and soybean meal. Waldroup *et al.* (2005, 2006) noted little or no improvement in metabolizable energy of soybean meal as the result of the addition of an α-galactosidase enzyme. Min *et al.* (2009) reported that two commercial enzyme products failed to improve energy digestibility of corn-soybean meal diets with and without the presence of 30% DDGS, even when fed at two and four times the recommended level.

Detection of response to enzyme supplementation is very subjective. If a diet already has a surfeit of nutrients, it is difficult to actually determine if an improvement has been made in nutrient utilization. It is commonly recommended that the diets be reduced in nutrient content when adding an exogenous enzyme; however in such a situation it is important to determine performance on the diet with reduced nutrients both with and without the supplemental enzyme. The present study was conducted to evaluate the effects of a commercial enzyme mixture on performance of broilers fed diets with and without a high level of DDGS.

MATERIALS AND METHODS

Rovabio Max™ is described by the supplier (Adisseo, Alpharetta GA) as a feed additive containing a naturally compatible combination of NSP enzyme activities produced by the non-genetically modified fungus *Penicillum funiculosum* combined with a 6-phytase produced from *Schizosaccharomyces pombe*. The enzymes contained in the product are said to include xylanases, β-glucanases, pectinases, mannanases and phytase as well as an α-galactosidase. A sample was obtained from the manufacturer and kept in a cool environment until use.

Diets were formulated following NRC (1994) recommendations for broilers 0-3 and 3-6 weeks of age with an adjustment in the lysine content. For the starter period, the lysine requirement was adjusted to 1.20% while for the grower phase it was adjusted to 1.10%. Diets were formulated with or without the addition of 20% DDGS of known protein and moisture content using matrix values reported by Waldroup *et al.* (2007). Three different nutrient levels were used in the formulation. These included: 1) "full requirements" as suggested by NRC; 2) reduced energy (-40 kcal/lb ME), available P (-0.12%) and calcium (-0.10%) indicated as EPC; 3) as 2 plus a 5% reduction in amino acid requirements (EPC+AA). All diets were fortified with complete vitamin and trace mineral mixes obtained from commercial sources. The combination of two basal diets fed at three nutrient levels resulted in six dietary treatments. Composition and calculated nutrient content of diets for 0-3 and 3-6 weeks are shown in Table 1 and 2, respectively. Each of these six diets was mixed and aliquots treated with Rovabio Max™ liquid at the recommended level of 200 g/ton for a total of twelve final treatments. The enzyme was preblended with a portion of the diet in a pharmaceutical-type V-mixer before blending with the remainder of the diet. Diets were pelleted with steam with crumbles fed for 0-21 d. Each of the twelve final treatments was assigned to four replicate pens of 60 male broilers each. The replicate pens were randomized in four quadrants of the house.

Male chicks of a commercial broiler strain (Cobb 500) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. New softwood shavings served as litter over concrete floors. Sixty chicks were assigned to each of 48 pens in a broiler house of commercial design. Each pen was equipped with two tube feeders and an automatic water font. Supplemental feeders and waterers were used during the first seven days. Temperature and airflow were controlled by automatic heaters and ventilation fans. Incandescent lights supplemented natural daylight to provide 23 hr of light daily. Care and management of the birds followed recommended guidelines (FASS, 1999). All procedures were approved by the University of Arkansas Institutional Animal Use and Care Committee.

Body weight and feed consumption by pen were determined at 21 and 42 d of age. At 21 and 42 d, two representative birds per pen were killed by CO_2 inhalation as recommended by the AVMA (1993) and tibias removed for bone ash determination as described by Yan *et al.* (2005). At 42 d, five representative birds per pen were processed as described by Fritts and Waldroup (2006) to determine dressing percentage and parts yield. Samples of all diets were assayed for crude protein, Ca, P and Na and were found to be in good agreement with calculated values.

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA using the General Linear Models procedure of the SAS Institute (1991). When significant differences among treatments were found, means were separated using repeated t-tests using the LSMEANS option of the GLM procedure. Main effects of nutrient level, DDGS inclusion and Rovabio Max™ inclusion were examined along with all two-way and three-way interactions. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers. Statements of statistical significance are based on $p<0.05$ unless otherwise noted.

Table 1: Composition (g/kg) and calculated nutrient content of diets with normal and reduced nutrient levels formulated with and without Distillers Dried Grains with Solubles (DDGS) for 0 to 3 wk of age. Values in bold are at minimum specified level

	Corn-Soy			DDGS		
Ingredients	FULL[a]	EPC[b]	EPC+AA[c]	FULL	EPC	EPC+AA
Yellow corn	610.42	637.83	666.87	458.85	484.48	509.05
Soybean meal	305.56	301.10	276.15	247.39	244.57	223.67
Poultry oil	42.73	22.44	18.19	52.90	32.90	29.32
DDGS	0.00	0.00	0.00	200.00	200.00	200.00
Limestone	5.04	8.33	8.59	9.49	12.75	13.02
Defluorinated phosphate	18.06	11.30	11.43	14.24	7.47	7.58
Sodium chloride	3.66	4.42	4.41	3.21	3.97	3.96
MHA-84[1]	2.83	2.79	2.53	1.87	1.81	1.51
L-Threonine	0.62	0.63	0.61	0.20	0.18	0.10
L-Lysine HCl	2.08	2.16	2.22	2.85	2.87	2.79
Vitamin premix[2]	5.00	5.00	5.00	5.00	5.00	5.00
Coban 90[3]	0.50	0.50	0.50	0.50	0.50	0.50
Mintrex P_Se[4]	1.00	1.00	1.00	1.00	1.00	1.00
Pel-Stik[5]	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
ME kcal/lb	**1450.00**	**1410.00**	**1410.00**	**1450.00**	**1410.00**	**1410.00**
Crude protein %	19.44	19.45	18.53	20.43	20.90	20.11
Calcium %	**1.00**	**0.90**	**0.90**	**1.00**	**0.90**	**0.90**
Nonphytate P %	**0.45**	**0.33**	**0.33**	**0.45**	**0.33**	**0.33**
Sodium %	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Met %	0.58	0.58	0.55	0.54	0.54	0.50
Lys %	**1.20**	**1.20**	**1.14**	**1.20**	**1.20**	**1.14**
Trp %	0.23	0.23	0.21	0.23	0.23	0.22
Thr %	**0.80**	**0.80**	**0.76**	**0.80**	**0.80**	**0.76**
Ile %	**0.80**	**0.80**	0.76	0.84	0.84	0.80
Val %	**0.90**	**0.90**	**0.86**	0.98	0.98	0.94
Leu %	1.67	1.68	1.62	1.92	1.93	1.88
Arg %	1.29	1.29	1.21	**1.25**	**1.25**	**1.19**
TSAA %	**0.90**	**0.90**	**0.86**	**0.90**	**0.90**	**0.86**

[a]Full = Full requirements; [b]EPC = Energy reduced 40 kcal/lb, available P reduced 0.12% and Ca reduced 0.10%, [c]EPC+AA = As EPC with 5% reduction in essential amino acid requirements.

[1]Methionine hydroxy analogue calcium salt. Novus International, St. Louis MO 63141.

[2]Provides per kg of diet: vitamin A 7715 IU; cholecalciferol 5511 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

[3]Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN 46825.

[4]Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 40 mg; Zn (as zinc methionine hydroxy analogue complex) 40 mg; Cu (as copper methionine hydroxy analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg. Novus International, Inc., St. Louis MO 63141.

[5]Uniscope Inc., Johnstown CO 80534

Table 2: Composition (g/kg) and calculated nutrient content of diets with normal and reduced nutrient levels formulated with and without Distillers Dried Grains with Solubles (DDGS) for 3 to 6 wk of age. Values in bold are at minimum specified level

	Corn-Soy			DDGS		
Ingredients	FULL[1]	EPC[1]	EPC+AA[a]	FULL	EPC	EPC+AA
Yellow corn	663.01	689.02	714.69	518.67	544.30	565.74
Soybean meal	262.75	259.55	237.56	197.99	195.16	176.92
Poultry oil	36.06	16.00	12.25	45.14	25.13	22.05
DDGS	0.00	0.00	0.00	200.00	200.00	200.00
Limestone	8.07	11.35	11.58	12.53	15.81	16.01
Defluorinated phosphate	12.75	5.98	6.10	8.97	2.20	2.29
Sodium chloride	4.28	5.04	5.04	3.83	4.59	4.59
MHA-84[1]	1.17	1.12	0.89	0.29	0.23	0.04
L-Threonine	0.68	0.67	0.60	0.37	0.35	0.22
L-Lysine HCl	2.23	2.27	2.29	3.21	3.23	3.14
Vitamin premix[1]	5.00	5.00	5.00	5.00	5.00	5.00
Coban 90[1]	0.50	0.50	0.50	0.50	0.50	0.50
Mintrex P_Se[1]	1.00	1.00	1.00	1.00	1.00	1.00

Tabel 2 Cont.:

	Corn-Soy			DDGS		
Ingredients	FULL[1]	EPC[1]	EPC+AA[a]	FULL	EPC	EPC+AA
Pel-Stik[1]	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
ME kcal/lb	**1450.00**	**1410.00**	**1410.00**	**1450.00**	**1410.00**	**1410.00**
Crude protein %	17.84	17.90	17.08	19.01	19.08	18.39
Calcium %	**0.90**	**0.80**	**0.80**	**0.90**	**0.80**	**0.80**
Nonphytate P %	**0.35**	**0.23**	**0.23**	**0.35**	**0.23**	**0.23**
Sodium %	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Met %	0.42	0.42	0.39	0.39	**0.38**	**0.36**
Lys %	**1.10**	**1.10**	**1.04**	**1.10**	**1.10**	**1.04**
Trp %	0.21	0.21	0.20	0.20	0.20	0.19
Thr %	**0.74**	**0.74**	**0.70**	**0.74**	**0.74**	**0.70**
Ile %	**0.73**	**0.73**	**0.69**	0.75	0.75	0.71
Val %	**0.82**	**0.82**	**0.78**	0.89	0.89	0.86
Leu %	1.56	1.57	1.52	1.79	1.81	1.76
Arg %	1.16	1.16	1.09	**1.10**	**1.10**	**1.04**
TSAA %	**0.72**	**0.72**	**0.68**	**0.72**	**0.72**	**0.68**

[1]As given in Table 1

Table 3: Effect of Rovabio Max on body weight of birds fed with and without DDGS and formulated to different levels of nutrient reduction. Means of four replicate pens of 60 males each

		21 d Body weight (kg)			42 d Body weight (kg)		
Nutrient level	Rovabio	Corn Soy	DDGS	Mean	Corn Soy	DDGS	Mean
Full	-RO	0.823	0.861	0.842	2.702	2.704	2.703
	+RO	0.887	0.837	0.862	2.724	2.669	2.697
	Mean	0.855	0.849	0.852^{ab}	2.713^{ab}	2.687^{ab}	2.700^{a}
EPC	-RO	0.854	0.882	0.868	2.612	2.673	2.642
	+RO	0.868	0.879	0.874	2.682	2.714	2.698
	Mean	0.861	0.881	0.871^{a}	2.647^{b}	2.693^{ab}	2.670^{ab}
EPC + AA	-RO	0.810	0.851	0.830	2.518	2.719	2.618
	+RO	0.804	0.869	0.837	2.521	2.730	2.625
	Mean	0.807	0.860	0.833^{b}	2.519^{c}	2.725^{a}	2.622^{b}
All diets	-RO	0.829	0.865	0.847	2.610	2.699	2.655
	+RO	0.853	0.862	0.857	2.642	2.704	2.673
	Mean	0.841^{y}	0.863^{x}		2.626^{y}	2.702^{x}	

	Prob > F	
Factor	21 d	42 d
Nutrient level (NUTR)	0.03	0.02
Full vs EPC	0.18	0.26
Full vs EPC+AA	0.18	0.01
EPC vs EPC+AA	0.01	0.07
Rovabio (ROVA)	0.35	0.38
DDGS	0.05	0.01
NUTR * ROVA	0.84	0.46
NUTR * DDGS	0.12	0.01
ROVA * DDGS	0.23	0.54
NUTR*ROVA*DDGS	0.14	0.82
CV	4.56	2.67

[abc]Means in columns with common superscripts do not differ significantly ($p<0.05$).

[xy]Means in rows with common superscripts do not differ significantly ($p<0.05$)

RESULTS

The effects of enzyme supplementation on diets with reduced nutrient levels with and without DDGS on body weight of male broiler chicks are shown in Table 3. At both 21 and 42 d of age, body weight was significantly influenced by the nutrient level of the diet. Compared to birds fed the full nutrient level, based on NRC (1994) recommendations with adjustment to the lysine content, birds fed the reduced levels of metabolizable energy, calcium, available P and amino acids (EPC+AA) had significant reduction in 42 d body weight but not at 21 d; however, birds fed diets with reduced levels of ME, Ca and available P only (EPC) did not differ in 21 or 42 d BW from those fed the full nutrient level. Moreover, the birds fed the EPC diet were significantly heavier at 21 d than those fed the EPC+AA diet, indicating that the reduced levels of amino acids had a more severe effect on BW than did the reductions in ME, Ca and P.

Inclusion of 20% DDGS in the diets did not have an adverse effect on BW at 21 or 42 d; in fact birds fed diets with the DDGS actually had significantly greater BW at both ages than birds fed diets without DDGS. Diets were formulated based on matrix values representing a composite of literature reports (Waldroup *et al*., 2007) and while the crude protein content of the sample used in the study was similar to the value used in formulation the digestibility of the amino acids and phosphorus was probably greater, as suggested by the light color and fresh odor of the product. There was a significant interaction of DDGS inclusion and nutrient level for 42 d body weight; birds fed the diets formulated to contain reduced levels of ME, Ca, P and amino acids (EPC+AA) with 20% DDGS were significantly heavier than those fed diets without DDGS (Fig. 1). Again, this may have been due to greater amino acid digestibility in the DDGS sample than assigned in the matrix values. The addition of Rovabio Max™ enzyme to the diets had no significant effects on body weight at 21 or 42 d and there were no significant two-way or three-way interactions of the enzyme with nutrient level or DDGS inclusion.

The effects of enzyme supplementation on diets with reduced nutrient levels with and without DDGS on feed conversion by male broiler chicks are shown in Table 4. Birds fed the diets with reduced nutrient levels (EPC and EPC+AA) had numerically reduced feed conversion at 21 d compared to the birds fed diets with full nutrient levels

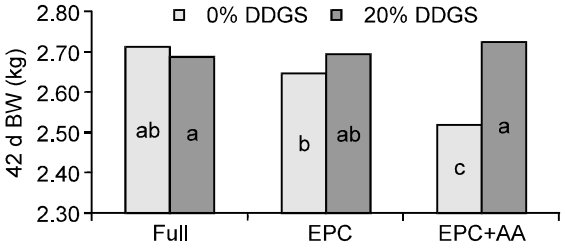

Fig. 1: Interaction of nutrient level and distillers dried grains with solubles (DDGS) on 42 d body weight of male broilers

(p = 0.16 and p = 0.18 respectively) and also at 42 d (p = 0.10 and p = 0.12 respectively). Based on the 40 kcal/lb reduction in ME between the diets with full and reduced nutrient levels, an increase in feed conversion of approximately 2.7% was anticipated but in actuality an increase of approximately 1.3% was observed. There was little difference in feed conversion between birds fed diets with reduced ME, Ca and P and those fed diets with reduced levels of ME, Ca, P and amino acids. Supplementing diets with Rovabio Max™ did not significantly improve feed conversion and in fact had significantly higher feed conversion at 21 d than did birds fed unsupplemented diets. There were no significant two-way or three-way interactions among the various factors for feed conversion.

Table 4: Effect of Rovabio Max on feed conversion rate of birds fed diets with and without DDGS and formulated to different levels of nutrient reduction. Means of four replicate pens with 60 birds each

		0-21 d Feed conversion (feed/gain)			0-42 d Feed conversion (feed/gain)		
Nutrient level	Rovabio	Corn Soy	DDGS	Mean	Corn Soy	DDGS	Mean
Full	-RO	1.528	1.545	1.537	1.723	1.707	1.715
	+RO	1.578	1.579	1.578	1.757	1.716	1.737
	Mean	1.553	1.562	1.557	1.740	1.712	1.726
EPC	-RO	1.548	1.518	1.533	1.752	1.736	1.744
	+RO	1.590	1.591	1.591	1.746	1.761	1.753
	Mean	1.569	1.555	1.562	1.749	1.748	1.749
EPC + AA	-RO	1.576	1.568	1.572	1.761	1.746	1.753
	+RO	1.564	1.600	1.582	1.735	1.748	1.742
	Mean	1.570	1.584	1.577	1.748	1.747	1.747
All diets	-RO	1.551	1.544	1.547[b]	1.746	1.729	1.737
	+RO	1.577	1.590	1.584[a]	1.746	1.742	1.744
	Mean	1.564	1.567		1.746	1.736	

	Prob > F	
Factor	0-21	0-42
Nutrient level (NUTR)	0.37	0.19
Full vs EPC	0.16	0.10
Full vs EPC+AA	0.18	0.12
EPC vs EPC+AA	0.28	0.92
Rovabio (ROVA)	0.01	0.58
DDGS	0.81	0.37
NUTR * ROVA	0.23	0.48
NUTR * DDGS	0.56	0.53
ROVA * DDGS	0.42	0.61
NUTR*ROVA*DDGS	0.55	0.54
CV	2.41	2.17

[abc]Means in columns with common superscripts do not differ significantly ($p<0.05$)

Table 5: Effect of Rovabio Max on mortality of birds fed diets with and without DDGS and formulated to different levels of nutrient reduction. Means of four replicate pens of 60 birds each

		0-21 d Mortality (%)			0-42 d Mortality (%)		
Nutrient level	Rovabio	Corn Soy	DDGS	Mean	Corn Soy	DDGS	Mean
Full	-RO	4.33	2.08	3.20	9.66	6.25	7.95
	+RO	2.22	2.08	2.15	8.33	8.33	8.33
	Mean	3.27	2.08	2.68	9.00	7.29	8.14
EPC	-RO	2.50	2.50	2.50	9.16	7.50	8.33
	+RO	2.77	1.66	2.22	11.25	5.83	8.54
	Mean	2.63	2.08	2.36	10.20	6.66	8.43
EPC + AA	-RO	1.25	1.66	1.45	7.08	7.50	7.29
	+RO	2.91	1.66	2.29	8.33	5.83	7.08
	Mean	2.08	1.66	1.87	7.70	6.66	7.18
All diets	-RO	2.69	2.08	2.38	8.63	7.08	7.86
	+RO	2.63	1.80	2.22	9.30	6.66	7.98
	Mean	2.66	1.94		8.97[x]	6.87[y]	

	Prob > F	
Factor	0-21 d	0-42 d
Nutrient level (NUTR)	0.47	0.54
Full vs EPC	0.63	0.80
Full vs EPC+AA	0.22	0.42
EPC vs EPC+AA	0.46	0.29
Rovabio (ROVA)	0.76	0.90
DDGS	0.19	0.04
NUTR * ROVA	0.35	0.97
NUTR * DDGS	0.82	0.55
ROVA * DDGS	0.83	0.57
NUTR*ROVA*DDGS	0.31	0.26
CV (transformed means)	0.88	1.52

[xy]Means in rows with common superscripts do not differ significantly ($p<0.05$)

Table 6: Effect of Rovabio Max on tibia ash of male broilers fed diets with normal or reduced nutrient levels with or without 20% Distillers Dried Grains with Solubles (DDGS). Means of four replicate pens with two birds each

		21 d Tibia ash (%)			42 d Tibia ash (%)		
Nutrient Level	Rovabio	Corn Soy	DDGS	Mean	Corn Soy	DDGS	Meal
Full	-RO	40.40	40.96	40.68	41.26	40.73	40.99
	+RO	41.03	42.05	41.53	40.27	41.00	40.64
	Mean	40.71	41.51	41.11[a]	40.77	40.87	40.82
EPC	-RO	41.25	38.90	40.07	40.78	41.34	41.06
	+RO	41.25	42.83	42.04	40.27	39.52	39.89
	Mean	41.25	40.87	41.06[a]	40.53	40.43	40.48
EPC + AA	-RO	38.42	39.18	38.80	39.03	39.43	39.23
	+RO	38.89	41.05	39.97	40.70	40.29	40.49
	Mean	38.65	40.11	39.38[b]	39.87	39.86	39.86
All diets	-RO	40.02	39.68	39.85[y]	40.36	40.50	40.43
	+RO	40.39	41.98	41.18[x]	40.42	40.27	40.34
	Mean	40.21	40.83		40.39	40.39	

	Prob > F	
Factor	21 d	42 d
Nutrient level (NUTR)	0.07	0.44
Full vs EPC	0.94	0.65
Full vs EPC+AA	0.04	0.20
EPC vs EPC+AA	0.05	0.41
Rovabio (ROVA)	0.05	0.89
DDGS	0.36	0.99
NUTR * ROVA	0.78	0.26
NUTR * DDGS	0.53	0.99
ROVA * DDGS	0.16	0.81
NUTR*ROVA*DDGS	0.55	0.66
CV	8.09	7.39

[ab, xy]Means in columns with common superscripts do not differ significantly ($p\leq0.05$)

Table 7: Effect of Rovabio Max on dressing percentage and parts yield of male broilers fed diets with normal or reduced nutrient levels with or without 20% Distillers Dried Grains with Solubles (DDGS). Means of four replicate pens with five birds each

		Dressing percent			Breast meat % of carcass			Leg quarters % of carcass			Wings % of carcass		
Nutrient level	Rovabio	CS	DDGS	Mean	CS	DDGS	Mean	CS	DDGS	Mean	CS	DDGS	Mean
Full	-RO	75.20	75.30	75.30	30.91^{xy}	30.34^{xy}	30.63	28.95	29.03	28.99	10.22	10.25	10.24
	+RO	74.70	75.30	75.00	31.12^{xy}	30.32^{xy}	30.72	28.85	28.35	28.60	10.38	10.67	10.52
	Mean	75.00	75.30	75.20	31.01	30.33	30.67	28.90	28.69	28.79	10.30	10.46	10.38
EPC	-RO	75.80	74.90	75.40	29.82^{y}	31.29^{x}	30.56	29.72	29.02	29.37	10.33	10.36	10.35
	+RO	75.00	75.40	75.20	30.91^{xy}	29.91^{y}	30.41	28.71	28.78	28.75	10.08	10.49	10.28
	Mean	75.40	75.10	75.30	30.36	30.60	30.48	29.22	28.90	29.06	10.21	10.42	10.32
EPC + AA	-RO	74.50	74.90	74.70	30.84^{xy}	30.77^{xy}	30.80	28.56	28.70	28.63	10.12	10.00	10.06
	+RO	75.70	74.80	75.30	29.83^{y}	31.00^{xy}	30.41	28.46	27.96	28.21	10.29	10.32	10.30
	Mean	75.10	74.80	75.00	30.33	30.88	30.61	28.51	28.33	28.42	10.21	10.16	10.18
All Diets	-RO	75.20	75.00	75.10	30.52	30.80	30.66	29.08	28.92	29.00	10.23	10.20	10.21^{b}
	+RO	75.20	75.20	75.20	30.62	30.41	30.51	28.68	28.36	28.52	10.25	10.49	10.37^{a}
	Mean	75.20	75.10		30.57	30.60		28.88	28.64		10.24	10.35	

	Prob > F			
Factor	Dressing %	Breast	Leg quarters	Wings
Nutrient level (NUTR)	0.63	0.88	0.09	0.11
Full vs EPC	0.72	0.57	0.35	0.47
Full vs EPC+AA	0.58	0.85	0.20	0.04
EPC vs EPC+AA	0.36	0.71	0.03	0.16
Rovabio (ROVA)	0.81	0.58	0.04	0.04
DDGS	0.83	0.92	0.30	0.12
NUTR * ROVA	0.45	0.79	0.93	0.12
NUTR * DDGS	0.62	0.18	0.96	0.37
ROVA * DDGS	0.77	0.35	0.75	0.08
NUTR*ROVA*DDGS	0.19	0.03	0.38	0.81
CV	2.75	6.72	6.04	5.42

$^{ab, xy}$Means with common superscript do not differ significantly ($p<0.05$). CS = Corn Soy

The effects of enzyme supplementation on diets with reduced nutrient levels with and without DDGS on mortality by male broiler chicks are shown in Table 5. Birds fed diets with 20% DDGS had significantly lower mortality at 42 d than birds fed the diets without DDGS. No other factor demonstrated any indication of a significant difference. Considering the high variability in mortality in studies with limited numbers of birds, this finding should be interpreted with caution.

The effects of enzyme supplementation on diets with reduced nutrient levels with and without DDGS on tibia ash of male broiler chicks are shown in Table 6. At 21 d, birds fed the diets with reduced levels of ME, Ca, P and amino acids had tibia ash that was significantly less than birds fed the diets with full nutrient levels and birds fed diets with reduced levels of ME, Ca and P. There was no significant difference in 21 d tibia ash between birds fed the full nutrient level and those fed the diet with reduced levels of ME, Ca and P. Inclusion of Rovabio Max™ to the diets significantly improved tibia ash at 21 d, probably due to the phytase activity present in this product. At 42 d, there were no significant differences in tibia ash related to any factor.

The effects of enzyme supplementation on diets with reduced nutrient levels with and without DDGS on processing characteristics of male broiler chicks is shown in Table 7. There were no significant dietary effects on dressing percentage. For breast yield there was a significant three-way interaction of nutrient level, DDGS inclusion and supplementation with Rovabio Max™; however there was no consistent pattern of response in this interaction. Birds fed Robabio Max™ had a significantly lower percentage of leg quarters and a significantly higher percentage of wings than birds fed diets without this enzyme.

DISCUSSION

Exogenous enzymes have long been effectively used in diets containing barley (Jensen *et al*., 1957; Fry *et al*., 1958; Willingham *et al*., 1959; Arscott and Rose, 1960). Some have noted improvements in corn-soybean meal diets with the addition of certain enzymes (Parkany-Gyarfas, 1975; Parkany-Gyarfas and Toth, 1978; Vogt and Harnish, 1978; Reese *et al*., 1983) while others have seen little or no improvement (Waldroup *et al*., 2005, 2006). In a review article dealing with the use of Rovabio® Excel in corn-soybean based diets, the use of Rovabio® Excel resulted in an increase in body weight and egg mass of 1.6, 1.6, 3.9 and 3.7% respectively for an unspecified number of broilers, laying hens, turkeys and ducks, with a reduction of 2.5, 2.0, 2.5 and 1.6% in feed conversion (Dalibard *et al*., 2004). West *et al*. (2007) added Rovabio® Excel to corn-soybean meal type diets differing in amino acid and energy

composition. Addition of the enzyme did not affect the broiler live performance or carcass traits, but did decrease 14-d mortality in one study and 41-d mortality in a second study. Rovabio® Excel had favorable effects when added to barley-based diets (Guerreiro *et al*., 2008) but not when added to diets high in sunflower meal (Mushtaq *et al*., 2006). Addition of Rovabio® improved the performance of broiler chickens fed low-density corn-soybean meal diets (Toledo *et al*., 2007). Addition of Rovabio® Excel AP to diets resulted in an increase in average body weight by 7% and a decrease of 3% in feed conversion ratio compared to control diets (Stupeliene *et al*., 2004). Min *et al*. (2009) observed no improvement in energy digestibility of either corn-soybean meal diets or diets high in DDGS from the addition of Rovabio Excel.

Demonstrating a response to an exogenous enzyme typically demands that the diets be deficient in some specific nutrient. In the present study, two levels of nutrient reduction were imposed. In the first level of reduction (EPC), ME was reduced by 40 kcal/lb, total Ca by 0.10% and available P by 0.12% compared to NRC (1994) recommendations with an adjustment in Lysine content. In the second level (EPC+AA), the same reductions were imposed along with a 5% reduction in total amino acid content. Birds fed the EPC diets did not differ significantly from those fed the positive control diet for body weight, feed conversion, mortality, tibia ash, dressing percentage, or breast meat yield at any point in the study, although numerical differences in feed conversion were observed. Additional reduction in amino acid content of the diets (EPC+AA) resulted in a significant decrease in 42 d body weight and a reduction in 21 d tibia ash compared to birds fed the positive control diet. Skinner *et al*. (1991) have reviewed the influence of dietary amino acids on bone formation. In addition, numerical differences in feed conversion were observed, but not commensurate to the reduction in dietary energy content. Therefore, response to any exogenous enzyme might be expected to be minimal. In the present study, the only positive responses to the addition of Rovabio Max were a significant improvement in 21 d tibia ash, probably due to the phytase activity of the enzyme combination and a significant increase in wing yield.

Inclusion of 20% DDGS in diets in the present study had no adverse effects on broiler performance; in fact body weights at 21 and 42 d were significantly higher in birds fed diets with DDGS. Diets in the present study were formulated using a nutrient matrix for DDGS derived from a compilation of literature values (Waldroup *et al*., 2007). The response to the DDGS was significantly higher in diets with reduced amino acid levels; the DDGS sample in the present study contained similar levels of crude protein but the quality of the product appeared to be superior as judged by the light color indicating minimal overheating. Several studies have indicated that broilers can effectively utilize diets with 20% DDGS provided adequate nutrient values are assigned to the product and diets are fed in a pelleted form (Waldroup *et al*., 1981; Wang *et al*., 2007a, 2007b, 2007c; Wang *et al*., 2008). The results of the present study are in full agreement with these findings.

In conclusion, there was little response to the addition of Rovabio Max to diets with normal or reduced nutrient levels. It would appear that the level of nutrient reduction in the present study was not sufficient to result in significant reduction in performance, other than tibia ash at 21 d in which case the addition of the Rovabio Max resulted in increased tibia ash. In order to demonstrate positive effects from the enzyme, it is probably necessary to have diets with a greater degree of nutrient reduction than imposed in the present study.

REFERENCES

Acamovic, T., 2001. Commercial application of enzyme technology for poultry production. World's Poult. Sci. J., 57: 225-242.

American Veterinary Medical Association (AVMA), 1993. Report of the AVMA panel on euthanasia. J. Am. Vet. Med. Assoc., 202: 230-249.

Arscott, B.H. and R.J. Rose, 1960. Use of barley in high-efficiency broiler rations. 4. Influence of amylolytic enzymes on efficiency of utilization, water consumption and litter condition. Poult. Sci., 39: 93-95.

Bedford, M.R. and A.J. Morgan, 1996. The use of enzymes in poultry diets. World's Poult. Sci. J., 52: 61-68.

Chesson, A., 2001. Non-starch polysaccharide degrading enzymes in poultry diets: Influence of ingredients on the selection of activities. World's Poult. Sci. J., 57: 251-253.

Dalibard, P. di, P.A. Geraer, M. Balasini and E. Vincenzi, 2004. Effects of a multiple enzyme preparation in a maize soybean diet for poultry. Rivista di Avicoltura, 73: 46-48.

FASS, 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st Rev. Edn., Federation of Animal Science Societies, Savoy IL.

Fritts, C.A. and P.W. Waldroup, 2006. Modified phosphorus program for broilers based on commercial feeding intervals to sustain live performance and reduce total and water-soluble phosphorus in litter. J. Appl. Poult. Res., 15: 207-218.

Fry, R.E., J.B. Allred, L.S. Jensen and J. McGinnis, 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poult. Sci., 37: 373-375.

Guerreiro, C.I.P.D., L.M.A. Ferreira, J.P.B. Freire, C.M.G.A. Fonts, J.A.M. Prates, P.I.P. Ponte, T. Ribeiro, L. Falcao and M.M.S. Lordelo, 2008. Role of a family 11 carbohydrate-binding module in the function of a recombinant cellulose used to supplement a barley-based diet for broiler chickens. Br. Poult. Sci., 49: 446-454.

Hill, F.W., D.L. Anderson, R. Renner and L.B. Carew Jr., 1960. Studies on the metabolizable energy of grains and grain products for chickens. Poult. Sci., 39: 573-579.

Hill, F.W. and R. Renner, 1960. The metabolizable energy of soybean oil meals, soybean millfeeds and soybean hulls for the growing chick. Poult. Sci., 39: 579-583.

Honig, D.H. and J.J. Rakis, 1979. Determination of the total pepsin-pancreatin indigestible content (dietary fiber) of soybean products, wheat bran and corn bran. J. Agric. Food Chem., 27: 1262-1266.

Jensen, L.S., R.E. Fry, J.B. Allred and J. McGinnis, 1957. Improvement in the nutritional value of barley for chicks by enzyme supplementation. Poult. Sci., 36: 919-921.

Min, Y.N., F. Yan, F.Z. Liu, C. Coto and P.W. Waldroup, 2009. Effect of various dietary enzymes on energy digestibility of diets high in distillers dried grains with solubles for broilers. J. Appl. Poult. Res., 18: 734-740.

Mushtaq, T., M. Sarwar, G. Ahmad, M.U. Nusa and A. Jamil, 2006. The influence of exogenous multienzyme preparation and graded levels of digestible lysine in sunflower meal-based diets on the performance of young broiler chicks two weeks posthatching. Poult. Sci., 85: 2180-2185.

NRC, 1994. Nutrient requirements of poultry. 9th Rev. Edn., National Academy Press, Washington DC.

Parkany-Gyarfas, A., 1975. Utilization of alpha-amylase supplemented diets in turkey feeding tests. Acta Alimen., 4: 23-35.

Parkany-Gyarfas, A. and M. Toth, 1978. Feed utilization efficiency of enzyme-containing feeds in livestock raising. Part I. Alpha-amylase-containing feeds in the raising of broiler chickens. Acta Alimen., 7: 111-120.

Potter, L.M. and E.D. Matterson, 1960. Metabolizable energy of feed ingredients for chickens. Rep. 9. Conn. Agr. Exp. Sta. Program, New Haven CT.

Reese, G.L., R.S. Karg, B.I. Fancher and P.W. Waldroup, 1983. The use of supplemental enzymes in the diet of broiler chickens. Nutr. Rep. Int., 28: 919-929.

SAS Institute, 1991. SAS® User's Guide: Statistics. Version 6.03 edition. SAS Institute Inc., Cary, NC.

Sibbald, I.R. and S.J. Slinger, 1962. The metabolizable energy of materials fed to growing chicks. Poult. Sci., 41: 1612-1613.

Skinner, J.T., J.N. Beasley and P.W. Waldroup, 1991. Effects of dietary amino acid levels on bone development in broiler chickens. Poult. Sci., 70: 941-946.

Stupeliene, A.R., V. Sasyte, R. Gruzauskas and S. Danius, 2004. Comparative evaluation of nutritional value of compound feeds for using thermal treated peas and supplement of enzyme preparation Rovabio Excel AP. Vet. Ir. Zootech., 26: 65-68.

Toledo, G.S.P. de, P.T.C. Costa, J.H. Silva, M. Ceccantini and C. Polleto Jr., 2007. Broilers fed diets varying in energy and protein supplemented with a pool of enzymes. Ciencia Rural 37: 518-523.

Vogt, H. and S. Harnish, 1978. Proteases in poultry feeds. Arch. Geflugelkd., 42: 183-193.

Waldroup, P.W., C.A. Fritts, C.A. Keen and F. Yan, 2005. The effect of alpha-galactosidase enzyme with and without Avizyme 1502 on performance of broilers fed diets based on corn and soybean meal. Int. J. Poult. Sci., 4: 920-937.

Waldroup, P.W., C.A. Keen, F. Yan and K. Zhang, 2006. The effect of levels of α-galactosidase enzyme on performance of broilers fed diets based on corn and soybean meal. J. Appl. Poult. Res., 15: 48-57.

Waldroup, P.W., J.A. Owen, B.E. Ramsey and D.L. Whelchel, 1981. The use of high levels of distillers dried grains with solubles in broiler diets. Poult. Sci., 60: 1479-1484.

Waldroup, P.W., Z. Wang, C. Coto, S. Cerrate and F. Yan, 2007. Development of a standardized nutrient matrix for corn distillers dried grains with solubles. Int. J. Poult. Sci., 6: 478-483.

Wang, Z., S. Cerrate, C. Coto, F. Yan and P.W. Waldroup, 2007a. Utilization of distillers dried grains with solubles (DDGS) in broiler diets using a standardized nutrient matrix. Int. J. Poult. Sci., 6: 470-477.

Wang, Z., S. Cerrate, C. Coto, F. Yan and P.W. Waldroup, 2007b. Use of constant or increasing levels of distillers dried grains with solubles (DDGS) in broiler diets. Int. J. Poult. Sci., 6: 501-507.

Wang, Z., S. Cerrate, C. Coto, F. Yan and P.W. Waldroup, 2007c. Effect of rapid and multiple changes in level of distillers dried grains with solubles (DDGS) in broiler diets on performance and carcass characteristics. Int. J. Poult. Sci., 6: 725-731.

Wang, Z., S. Cerrate, C. Coto, F. Yan and P.W. Waldroup, 2008. Evaluation of high levels of distillers dried grains with solubles (DDGS) in broiler diets. Int. J. Poult. Sci., 7: 990-996.

West, M.L., A. Corzo, W.A. Dozier III, M.E. Blair and M.T. Kidd, 2007. Assessment of dietary Rovabio Excel in practical United States broiler diets. J. Appl. Poult. Res., 16: 313-321.

Willingham, H.E., L.S. Jensen and J. McGinnis, 1959. Studies on the role of enzyme supplements and water treatment for improving the nutritional value of barley. Poult. Sci., 38: 539-544.

Yan, F., C.A. Keen, K.Y. Zhang and P.W. Waldroup, 2005. Comparison of methods to evaluate bone mineralization. J. Appl. Poult. Res., 14: 492-498.

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