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Full Length Research Paper

Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks

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Two experiments were conducted to determine the effects of dietary supplementation of REAP® enzyme into corn-soybean diet on the energy utilization in poultry and performance of broiler chicks. In the first experiment, a total of 16 50 weeks adult roosters (ISA-Brown) were divided into 4 groups with 4 birds per replicate and the experimental diets contained the two levels of energy (2650 and 2759 kcal TMEn/kg diet) with 0 or 0.1% REAP® and were subjected to assay of apparent metabolizable energy (AME). In the second experiment, 360 3 days old male broiler chicks (Ross) were divided into 4 groups with 3 replicates of 30 birds per replicate and were assigned at random to one of the four experimental diets containing the two levels of energy (3100 and 2980 kcal TMEn/kg diet) with 0 or 0.1% REAP®. AME value of the high energy groups were significantly higher ($p < 0.05$) than that of the low energy groups when measured at 28 days. The body weight gain of the birds fed the low energy diet with 0% REAP was lower significantly than those of the other groups ($p < 0.05$). There were no significant differences in feed intake and feed conversion rate among the treatments. The breast muscle weights of the low energy diet birds were higher than those of the high ones and those of the lower energy group with 0.1% REAP were the highest ($p < 0.05$). The relative abdominal fat weight was reduced by the dietary REAP ($p < 0.05$). Percentage duodenum weights of high energy group were higher than those of the low energy group. The intestinal lengths (cm/100 g BW) of low energy diet group without REAP were lower than those of the others ($P < 0.05$). The results demonstrated that, dietary REAP improved body weight gain and reduced abdominal fats. Therefore, it can be concluded that, dietary supplementation of REAP improves nutritive value of corn-soybean diet in the broiler chicks.

Key words: REAP®, broiler chicks, corn-soybean, apparent metabolizable energy (AME).

INTRODUCTION

Corn-soybean meal poultry feed is considered to be favorable because of its high nutritional value but soybean meal contains oligosaccharides that have been shown to decrease bird health and growth (Iji and Tivey, 1998) and in corn cell wall, arabinoxylans, ß-glucan and cellulose are present. There is strong evidence that, some nutrients in corn are not completely digested in the small intestine and that considerate amounts of starch and protein escape digestion, reach the midgut and undergo fermentation with a relatively low energy yield (Noy and Sklan, 1995). Among potential factors reducing nutrient bioavailability are the non-starch polysaccharides (NSP). In most of the studies, with adding an enzyme or enzyme mixture to corn-soybean diets, a significant increase in NSP digestibility was observed, indicating that the enzymatic degradation of cell wall polysaccharides is possible despite the complex nature of these polymers.(Meng et al., 2005). Enzymes have been added to broiler diets for more than 30 years. Supplementing corn-soybean meal diets with an enzyme or enzyme mixture that possess a broad-spectrum range of activities may improve the digestibility and as a result, growth performance (Odetallah et al., 2002). Several studies have demonstrated some beneficial effect on the AME and NSP digestibility of soybean diets, depending on the

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Table 1. Ingredients of rations in different treatments (%).

Ingredient	High energy		Low energy	
	-	+	-	+
Corn	56.2	56.2	60.1	60.1
Soybean meal	34.5	34.5	33.2	33.2
Fish meal	3	3	2.8	2.8
Soybean oil	3.5	3.5	1.2	1.2
Dicalcium phosphate	1.3	1.3	1.2	1.2
Common salt	0.4	0.4	0.37	0.37
Mineral premix	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5
DL-methionine	0.11	0.11	0.12	0.12
TMEn (kcal)	3100	3100	2980	2980

(-) Indicates non-addition of REAP and (+) indicates the addition of 0.1% of REAP.

enzyme preparation used (Zhou et al., 2009; kocher et al., 2002; Fuente et al., 1995). Hesselman and Aman (1986) proposed that, ß-glucanase breaks down cell wall and releases nutrients from cellular contents of digestion. Recently, *in vitro* studies in a laboratory (Saleh et al., 2003a, b) showed that, digestibility was higher when protease was excluded from the mixture of enzymes. Zhou et al. (2009) reported that, supplementing a corn-soybean broiler starter diet with an enzyme preparation containing a mixture of xylanase, protease and amylase resulted in improvements in AME value of diets in the starter, grower and finisher phases. REAP® is a multi-enzyme with ß-glucanase, protease and cellulose activity. Therefore, this study was conducted to investigate whether REAP® could affect the AME and improve the performance of poultry.

MATERIALS AND METHODS

Two experiments were conducted to determine the effects of dietary supplementation of REAP® enzyme in corn-soybean diet on the energy utilization in poultry and performance of broiler chicks. The enzyme preparation used in this study was a commercial multi-enzyme complex with ß-glucanase, protease and cellulose. In the first experiment, a total of 16 50 weeks adult roosters (ISA-Brown) of uniform body weight (BW) (2.4 to 2.8 kg) were obtained from a commercial farm and divided into 4 groups with 4 birds per replicate and subjected to assay of apparent metabolizable energy (AME) (Farrell, 1978). They were housed in cages; 1 per cage that permitted collection of excreta on plastic. Experimental period was 28 days. The birds were fasted for 24 h before the introduction of assay diets. Four treatment groups which included high energy groups (with 2759 kcal/kg TMEn and 0% or 1% REAP®) and low energy groups (with 2650 kcal/kg TMEn and 0% or 1% REAP) were used.

All the samples of excreta were collected separately for each rooster. These were weighed, oven dried at 100℃ and ground to pass through 0.3 mm mesh sieve. The gross energy (GE) content of each feedstuff and its excreta was determined with the help of Parr adiabatic oxygen bomb calorimeter (Nukamp, 1965). Samples of feedstuffs tested for TME contents were subjected to proximate analysis (AOAC, 1990). The AME and TMEn per kg of feed were calculated by the following formula:

$$\text{AME (Kcal/kg)} = \frac{\text{GE intake- GE excreta}}{\text{Intake}} \times 1000 \quad (1)$$

$$\text{TMEn (Kcal/kg)} = \frac{\text{GE intake} - (\text{GE excreta} - \text{GE endogenous}) - 8.22 \times \text{nitrogen balance (g)}}{\text{Intake}} \times 1000 \quad (2)$$

In the second experiment,360 3 days old male broiler chicks (Ross) were divided into 4 groups with 3 replicates of 30 birds per replicate and were assigned at random to one of the four experimental diets containing the two levels of energy (3100 and 2980 kcal TMEn/kg diet) with 0 or 0.1% REAP® (Table 1). The birds were housed in an environmentally controlled room and feed and water were provided *ad libitum* from 3 to 23 days of age. Feed consumption, body weight gain and feed conversion rate were measured weekly. Body composition, the length and weight of the intestine (duodenum, jejunum, and ileum) were determined at 23 days of age.

Statistical analysis of the data as a completely randomized design was accomplished using the General Linear Models procedure of SAS (SAS Institute, 1990). Differences between means were determined using the least significant difference mean separation procedure.

RESULTS

The enzyme activity of α-galactosidase in REAP® was 13,708 unit/g. Although there were no significant differences, the AME value of the dietary supplementation of REAP® tended to be increased at the 14th day. The AME value of the high energy groups were significantly higher ($p < 0.05$) than the low energy groups when measured at the 28th day (Table 2). However, the dietary supplementation of REAP® did not affect AME values of the diets for the experimental period.

The body weight gain of birds fed the low energy diet with 0% REAP was lower significantly than the other groups ($p < 0.05$) (Table 3). There were no significant

Table 2. Effects of REAP® supplementation on metabolizable energy in roosters.

	High energy		Low energy	
	-[1]	+[1]	-	+
14 days	**kcal/kg**			
Gross energy	3643	3643	3513	3513
Fecal energy	2722	2592	2693	2333
AME	2922±38	2958±82	2792±20	2892±21
28 days				
Gross energy	3643	3643	3513	3513
Fecal energy	2349	2517	2674	2433
AME	2992±3[a]	2982±4[a]	2809±11[b]	2869±12[b]

[a,b]Means ±SE within a row with no common superscripts differ significantly ($P < 0.05$). [1] A (-) indicates non-addition of REAP, and a (+) indicates the addition of REAP.

Table 3. Effect of REAP supplementation on daily weight gain in broiler chicks.

	High energy		Low energy	
	-[1]	+[1]	-	+
	g/day/bird			
1 week	20.8±0.5	21.0±0.7	20.4±0.3	20.9±0.1
2 weeks	43.3±1.0[ab]	45.7±0.4[a]	41.04±0.0[b]	43.2±0.7[ab]
3 weeks	59.1±0.4[a]	58.1±0.8[ab]	55.7±0.6[b]	58.0±0.4[ab]
Mean	41.1±0.3[a]	41.5±0.4[a]	39.1±0.4[b]	40.6±0.1[a]

[a,b]Means± SE within a row with no common superscripts differ significantly ($P < 0.05$).[1] A (-) indicates non-addition of REAP and a (+) indicates the addition of REAP.

Table 4. Effect of REAP® supplementation on feed intake in broiler chicks.

	High energy		Low energy	
	-1	+[1]	-	+
	g/day/bird			
1 week	31.6±0.8	31.1±0.2	31.7±0.6	32.3±0.1
2 weeks	70.5±0.5	69.9±0.7	71.6±1.1	71.1±1.3
3 weeks	95.6±0.3[a]	91.8±0.7[b]	91.9±0.1[b]	93.2±0.8[ab]
Mean	65.9±0.5	64.7±0.7	65.06±0.4	65.4±0.2

[a,b]Means ± SE within a row with no common superscripts differ significantly ($P < 0.05$). [1] A (-) indicates non-addition of REAP® and a (+) indicates the addition of REAP®.

differences in the feed intake (Table 4) and feed conversion rate (Table 5) among the treatments.

The relative sizes (percent body weight) of the liver and leg muscle were not different significantly among the treatments. The breast muscle weights of the low energy diet birds were higher than those of the high ones and those of the lower energy group with 0.1% REAP were the highest. The relative abdominal fat weight was reduced by the dietary REAP (Table 6).

No significant differences in the relative jejunum and

Table 5. Effect of REAP supplementation on feed conversion rate in broiler chicks.

Week	High energy		Low energy	
	-	+	-	+
	Feed/gain			
1 week	1.52±0.01	1.49±0.04	1.55±0.05	1.54±0.01
2 weeks	1.63±0.05[ab]	1.53±0.00[b]	1.74±0.03[a]	1.65±0.06[ab]
3 weeks	1.62±0.02	1.58±0.03	1.65±0.02	1.61±0.02
Mean	1.62±0.02	1.58±0.03	1.65±0.02	1.61±0.02

[a,b]Means ± SE within a row with no common superscripts differ significantly ($P < 0.05$). (**-**) Indicates non-addition of endopower and a (**+**) indicates the addition of REAP.

Table 6. Effects of REAP supplementation on relative organ weights in broiler chicks.

Organ	High energy		Low energy	
	-	+	-	+
	g/100 g body weight (BW)			
Liver	2.55±0.03	2.82±0.10	2.64±0.11	2.68±0.06
Abdominal fat	1.73±0.08[a]	1.57±0.07[ab]	1.73±0.18[a]	1.17±0.14[b]
Breast muscle	5.53±0.15[bc]	5.32±0.10[c]	6.06±0.30[ab]	6.32±0.17[a]
Leg muscle	8.75±0.16	9.08±0.20	8.99±0.20	8.84±0.07

[a-c]Means ± SE within a row with no common superscripts differ significantly ($P < 0.05$). (**-**) Indicates non-addition of REAP and (**+**) indicates the addition of REAP.

Table 7. Effects of REAP supplementation on weight and length of small intestine in broiler chickens.

Parameter	High energy		Low energy	
	-	+	-	+
	g/100 g body weight (BW)			
Weight				
Duodenum	1.42±0.07[ab]	1.52±0.06[a]	1.22±0.07[b]	1.26±0.06[b]
Jejunum	1.58±0.10	1.90±0.17	1.64±0.05	1.62±0.08
Ileum	1.61±0.09	1.72±0.23	1.53±0.13	1.48±0.10
Length				
	cm/100g BW			
Duodenum	2.62±0.09[a]	2.54±0.06[a]	2.25±0.10[b]	2.58±0.11[a]
Jejunum	6.18±0.25[ab]	6.94±0.27[a]	5.52±0.29[b]	6.27±0.22[ab]
Ileum	5.84±0.25[ab]	6.50±0.55[a]	5.42±0.08[b]	5.81±0.17[ab]

[A,b]Means ± SE within a row with no common superscripts differ significantly ($P < 0.05$). (-) Indicates non-addition of REAP and (+) indicates the addition of REAP.

ileum weights were found, but percentage duodenum weights of high energy group were higher than those of the low energy group. The intestinal lengths (cm/100 g BW) of the low energy diet group without REAP were lower than those of the others (Table 7). The results demonstrated that, dietary REAP improved body weight gain and reduced abdominal fats. Therefore, it can be concluded that, dietary supplementation of REAP improved the nutritive value of the corn-soybean diet in the broiler chicks.

DISCUSSION

The body weight gain of birds fed the low energy diet with 0% REAP® was lower significantly than the other groups (p< 0.05) but among the groups, there were no significant differences in the feed conversion ratio. This result are in agreement with that reported by Greenwood et al. (2002) who showed that supplementation of a corn-soybean broiler starter diet with a mixture of xylanase, protease and amylase improved the BW at 14 and 42 days of age with no significant effects on the feed conversion ratio (FCR).

FCR of birds fed the low energy diet with 0% enzyme was higher than those of the other group, although, not significantly. Addition of the enzyme to the birds diet showed trend towards improving the BWG significantly (P < 0.05) and FCR numerically.

Pertilla et al. (2001) suggested that, the ß-glucans present in corn-soybean meal diet may have affected the digestibility of nutrients. A possible explanation for this could be additional ß-glucanase protease and cellulose activity contained in this enzyme preparation which might enhance the digestion of NSP and protein because by disruption of the cell wall, encapsulated intracellular nutrient may be released. Furthermore, ß-glucans has been shown to decrease ileal digesta viscosity (Almirall et al., 1995; Esteve-Garcia et al., 1997; Fuente et al., 1998). Some studies indicated that, inclusion of supplemental protease, α-amylase, ß-glucanases and mixed enzymes might have a positive influence on animal growth (Merstad and McNab, 1975: Moss et al., 1977; Pettersson and Aman, 1989) and increase the availability of nutrients (Walsh et al., 1993). Improvement is often attributed to the degradation of NSP (Odetallah, 2002) and improvement of the digestion and absorption of nutrients, such as amino acids and energy (Oloffs et al., 1999; Mathlouthi et al., 1999; Rutkowski et al., 1999), protein (Oloffos et al., 1999) and fats (Francesch et al., 1999). The development of secretion of digestive enzymes in the post hatched chick could also be a limiting factor in digestion (Krogdahl and Sell, 1989, Noy and Sklan, 1995; Sklan, 2001). A variety of complex proteins may not be easily digested by the young chick due to the rapid food rate and the deficiency of the necessary innate enzyme (Uni et al., 1999) leading to inefficient growth by birds, poor feed conversion ratio, and poor livability. The insufficient enzyme activity for early chicks may possibly be complemented through exogenous enzyme supplementation to promote digestion and utilization of diets. In contrast, Kocher et al. (2002) did not show significant improvements in the BWG of broilers by supplementing a commercial enzyme containing mainly hemicellulase, pectinase, ß-glucanase and some protease activities. Studies with similar multienzyme preparation (Energex) also failed to bring improvement in BWG (Mohamed and Hamza, 1991; Marsman et al., 1997)

It was shown that cellulose activity was significantly reduced after incubation with protease (Saleh et al., 2004). The lack of improvement in the performance reported by those researchers could be attributed to the protease activity in their enzyme preparations.

There were no significant differences in the feed intake among the groups. The addition of REAP® failed to show any significant effect on the feed intake. This result is in agreement with that reported by Tahir et al. (2005). After partial degradation, some of the insoluble NSP becomes soluble NSP with a high-molecular weight (Castanon et al. 1997). The high molecular weight NSP increases digesta viscosity (Chesson, 2001), which reduces the availability of nutrients by reducing the ingestinal passage (Campbell et al., 1989; Rotter et al., 1989; Choct and Annison.1992). However, Dänicke et al. (1999) reported that, feed intake was higher for enzyme supplemented diets, since use of enzyme decreases mean retention time of digesta in the gizzard and large intestine and increases gut motility. Digesta viscosity and microbial fermentation decrease nutrient digestibility and the rate of absorption are increased so that more feed can be eaten. Pertilla et al. (2001) reported that, the actual differences in feed intake were relatively minor with their effect on rate of passage and digestibility likely to be negligible. An increase in the carcass weight is a typical response to an increased calorie: protein ratio (Mabray and Waldroup, 1981; Donaldson et al., 1985). Sibbald (1980) showed that the variation in the TME values of the different foodstuffs was more dependent on the rate of passage.

Some studies reported an increase in the AME of a corn-soybean diet by supplementing commercial enzyme preparations (Zanella et al., 1999; Douglas et al., 2000; Kocher et al., 2002; Gracia et al., 2003) but in the present study, the dietary supplementation of REAP® did not affect the values of the diets. However, digestibility coefficients measured with older animals were not universally applicable for the optimization of young broiler diets (Pertilla et al., 2001). ß-glucans are less significant in older animals (Pettersson et al., 19991). Digestive enzyme activities (units/ kg of BW) measured in the pancrease and intestinal contents increased with age (Nitsan et al., 1991). In mature animals, the digestive tract is large and therefore, the rate of digesta passage is slower and enzymes and microbes have more time to digest food. Consequently, the effect of ß-glucan is expected to diminish and the digestibility of nutrients to improve with age (Almirall et al., 1995).

Results of a study by Pertilla et al. (2001) showed that, ß-glucans increased viscosity and decreased the apparent ileal nutrient digestibility (AID) of amino acid in broilers. A mixture of enzymes could increase the protein digestibility of broiler feed. The enzymes could also save dietary protein (Tahir et al., 2008). A protein-sparing effect by Versazyme (VZ) was suggested by Odetallah et al. (2005). Numerical improvements in the body weight and breast meat yield in broiler chickens was reported with enzyme supplementation to diets but most prominently when applied to low ME diets with calculated ME in the range of 2964 to 3185 kcal / kg (Sims et al., 2001).

In this study, the breast muscle weight of the low energy diet birds (2980 kcal / kg) with 0.1% REAP® was significantly higher than that of the high energy group without enzyme ($p < 0.05$). The abdominal fat was reduced significantly by the enzyme ($P < 0.05$). This result is in agreement with that reported by Tahir et al. (2005). The increase in abdominal fat was reported to be a typical response to an increase in energy: protein ration (Mabray and Waldroup, 1981; Donaldson, 1985). The degradation of cellulose into smaller molecules, such as cellobiose, by cellulose might affect intestinal microflora, thereby changing the lipid metabolism (Tahir et al., 2005). It has been reported that, alternations of colonic microflora influence serum lipid levels (Jenkins et al., 1999). Viscous polysaccharides cause physiological and morphological changes to the digestive system in various species (Brown et al., 1979; Cassidy et al., 1981; Jacobs, 1983).

In this study, the enzyme treatment did not affect the relative weights of the digestive system and liver. The results are in close agreement with those reported by Tahir et al. (2005) who showed that enzyme treatments (cellulose and hemicellulase) did not affect the relative weights of the digestive system and liver. Gracia et al. (2003) also reported that, enzyme has no effect on the relative weights of digestive organs. But the addition of enzyme to the low energy diet group increased the duodenum length significantly ($p < 0.05$). Duodenum plays an important role in nutrient absorption.

Silva and Smithard (2002) suggested that the absorption of nutrients may be impeded by an increase in the thickness of the unstirred layer in the small intestine. It may be concluded that the antinutritive effect of NSP is related to their ability to increase digesta viscosity which in turn causes changes in gut morphology and in the efficiency of nutrient utilization by the chicken (Mehri et al., 2010).

The data presented indicated that, dietary REAP improved body weight gain and reduced abdominal fats. Therefore, it can be concluded that, dietary supplementation of REAP improves nutritive value of corn-soybean diet in broiler chicks.

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