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RESEARCH ARTICLE

Supplementation of exogenous enzymes and organic acid in broiler diets

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Öz

Rahman A, Saima, Pasha TN, Akram M, Abbas Y, Ullah S. Broyler rasyonlarına ekzojen enzim ve organik asit ilavesi.

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Amaç: Bu araştırma düşük enerjili rasyonla beslenen broylerlerde organik asit varlığında enzim aktivitesinin etkinliğini değerlendirmek amacıyla yapıldı.

Gereç ve Yöntem: Araştırmada toplam 240 adet günlük yaşta Hubbard broyler civciv her biri 10 civciv içeren 3 alt gruptan oluşan 8 gruba ayrıldı. Buna göre deneme grupları temel rasyon A (pozitif kontrol, 2730 kcal/kg), düşük enerjili rasyon B (negatif kontrol, 2630 kcal/kg), düşük enerjili rasyon + 0.25 g/kg enzim içeren rasyon C, düşük enerjili rasyon + 0.5 mg/kg enzim içeren rasyon D, düşük enerjili rasyon + %0.5 sitrik asit içeren rasyon E, düşük enerjili rasyon + %1.5 sitrik asit içeren rasyon F, düşük enerjili rasyon + 0.25 g/kg enzim + %0.5 sitrik asit içeren rasyon G ve düşük enerjili rasyon + 0.5 g/kg enzim + %1.5 sitrik asit içeren rasyon H şeklinde oluşturuldu.

Bulgular: Araştırmada canlı ağırlığın deneme süresince D, G ve H gruplarında A'ya göre değişmezken (P>0.05), B'ye göre geliştiği (P<0.05) tespit edildi. Yem tüketiminin D ve G grupları ile A'da benzer iken (P>0.05), tüm gruplarda B'ye göre düşük olduğu (P<0.05) belirlendi. Yemden yararlanma oranının D ve H'de en iyi iken (P<0.05), metabolize olabilir enerjinin gruplarda değişiklik oluşturmadığı tespit edildi. Kan glikoz düzeyinin D, G ve H'de daha yüksek olduğu belirlendi.

Öneri: Düşük enerjili rasyonla beslenen broylerlerde enzim ilavesinin performansı geliştirirken, organik asit ilavesinin performans üzerine belirgin bir etki oluşturmadığı ifade edilebilir.

Anahtar kelimeler: Enzim, organik asit, canlı ağırlık, yemden yararlanma oranı, broyler

Abstract

Rahman A, Saima, Pasha TN, Akram M, Abbas Y, Ullah S. Supplementation of exogenous enzymes and organic acid in broiler diets.

Aim: This study was conducted to evaluate the efficiency of enzyme activity in the presence of organic acid in broiler fed low energy diet.

Materials and Methods: A total of 240 day-old Hubbard broiler chicks were reared in 8 groups with 3 replicates per group, comprising 10 birds in each replicate. The dietary treatment groups comprised of basal diet A (positive control, 2730 kcal/kg), low energy diet B (negative control, 2630 kcal/kg), diet C containing low energy diet plus 0.25 g/kg enzyme, diet D containing low energy diet plus 0.5 mg/kg enzyme, diet E containing low energy diet plus 0.5% citric acid, diet F containing low energy diet plus 1.5% citric acid, diet G containing low energy diet plus 0.25 g/kg enzyme plus 0.5% citric acid and diet H having low energy diet plus 0.5 g/kg enzyme plus 1.5% citric acid were offered to different groups of broiler.

Results: Results showed improved weight gain throughout the experiment (P<0.05) in groups D, G and H groups as compared to B while non-significant (P>0.05) as compared to A. Feed consumption was reduced (P<0.05) in all groups as compared to B while D and G was equal to A in feed consumption (P>0.05). Feed conversion ratio (FCR) was observed better (P<0.05) in D and H while M.E was non-significant (P>0.05) in all groups. Blood glucose level was observed higher (P<0.05) in D, G and H.

Conclusion: It may be stated that enzyme supplementation improves performance in broiler fed low energy diet while organic acid supplementation in low caloric diet has no pronounced effect on performance.

Keywords: Enzyme, organic acid, weight gain, feed conversion ratio, broiler

Introduction

Poultry farming is expanding to fulfill the ever increasing demand of human population with limited feed resources. Use of nonconventional feed resources may be an alternative to make the better utilization of nutrients in feed (Ravindran and Cyril 1995).

Feed cost is a major segment of farm expenses. It can be reduced by efficient utilization of nutrients in feed. Non starch polysaccharides (NSPs) cannot be digested by the endogenous enzymes as poultry lacks the NSPs digesting enzymes (Ao et al 2009). These components of feed remain undigested having some part of energy in them which is wasted. The use of exogenous NSPs digesting enzymes may be a good tool to break down these components and release some energy. Grains and legumes have α-galactosides (Raffinose, stachyos) and NSPs like hemicellulose, mannan, and raffinose (Veldman and Vahl 1994, Iirish et al 1995). Corn-soybean-based diets without supplemented enzymes such as xylanases and pectinases might result in gas accumulation in the gut and diarrhea (Jaroni et al 1999, Wu et al 2005). The accumulation NSPs may result in fluid retention and increased flow rate which negatively affects digestion and absorption of nutrients. Enzyme supplementation is considered to break the bond among NSPs and result in improved nutritional value of feed materials (Giraldo et al 2008, Balamurugan and Chandrasekaran 2009). The effect of exogenous enzyme may be variable as it is dependent on different factors like bird age, feed type and quality (Bedford 2000, Acamovic 2001).

Use of organic acids to replace antibiotics is a new trend in poultry nutrition. It enhances the growth and protects the birds from diseases as antibiotics have been banned in European countries. Organic acids also reduce the pH of the digesta (Dibner and Buttin 2002).

Supplementation of organic acid to diets has indicated positive results in broilers (Skinner et al 1991). These are bactericidal for Salmonella in crop and influence bacterial population in caecum and duodenum (Thompson and Hinton 1997). Furthermore addition of organic acids in diet is known to lower the pH of feed and digesta (Giesting and Easter 1985). The pH level usually found in corn-soy based diets is approximately 6.0 which is higher than the optimal pH level (4.5) for the proper functioning of α-galactosidase (Ademark et al 2001). Ao et al (2009) showed that in-vitro activity of α-galactosidase was significantly reduced as the pH was increased above 5.0. Complex enzyme cocktail, named Zympex® 008, is a multi-enzyme complex consisting of enzymes necessary for breakdown of fiber, starch, NSPs and proteins as α-galactosidase is a fungal origin enzyme and causes hydrolysis of the oligosaccharides, β-mannanase which hydrolyze the mannan part of carbohydrate, cellulase is class of enzymes produced mainly by fungi, bacteria, and protozoans that catalyze the hydrolysis of cellulose, amylase breaks starch into sugar, β-xylanase degrades the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicellulose, the major components of plant cell walls (Dashek 1997), β-glucanase catalyzes the α-glucosan in plants into glucose, so as to reduce the viscosity of digesta. Protease breaks down proteins by proteolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases work best in acidic conditions except alkaline proteases. Its optimal activity is shown in alkaline pH (Barrett et al 2003). In view of these findings, the current study was conducted to evaluate the effect of multi-enzyme and organic acid on growth performance and nutrient utilization in broilers when added in feed having low energy.

Materials and Methods

This study was conducted at Poultry Research Centre, University of Veterinary and Animal Sciences, Lahore. Abridgement of this experiment was planned taking into account all the national legislation concerning protection of animal welfare and following the guidelines of the Advanced Studies and Research Board (ASRB) of the university. A commercial enzyme source of multi-enzyme (NSP enzyme Zympex, Impextraco, Belgium) and feed-grade anhydrous citric acid was procured from local market. A total 240 day-old Hubbard broiler chicks of mixed gender were procured from commercial hatchery. The study lasted for 5 weeks. All birds were weighed individually at arrival (1-day old) and kept in fumigated experimental house under uniform managemental conditions. Experimental diet and clean water was offered ad libitum. All the managemental practices were followed according to standard schedule as per Hubbard guide lines.

Birds (n=240) were divided in eight groups. Eight experimental diets were formulated and were offered to eight groups randomly. Group A (Control group) was offered diet according to NRC (1994) recommended recommendations. Group B was offered diet with low energy (Control-2, 2630 kcal/kg), group C was offered diet containing low energy

Table 1. Dietary treatments.

Treatment groups	Composition
A	Normal basal diet (positive control)
B	Low energy diet (negative control)
C	Diet B + 0.25 g enzyme/kg feed
D	Diet B + 0.5 g enzyme/kg feed
E	Diet B + 0.5% citric acid
F	Diet B + 1.5% citric Acid
G	Diet B + 0.25 g enzyme/kg feed + 0.5% citric acid
H	Diet B + 0.5 g enzyme/kg feed + 1.5% citric acid

Table 2. Ingredient and chemical composition of experimental diets.

Ingredients	Diet A* (%)	Diet B** (%)
Corn	46.53	43.58
Rice polish	15.00	15.00
Canola meal 34%	11.16	12.00
Rape seed meal 35%	3.00	3.00
Soybean meal 46%	4.54	3.55
Sunflower meal 28%	0.00	4.51
Guar meal	4.0	4.00
Corn gluten meal 60%	1.27	1.61
Corn gluten meal 30%	2.50	2.50
Fish meal 52%	5.00	2.29
Molasses	4.00	4.00
Premix***	0.40	0.40
Chips	0.30	0.21
Bone meal	1.66	2.54
Salt	0.12	0.17
Sodium bicarbonate	0.10	0.10
L-Lysine	0.30	0.39
DL-Methionine	0.10	0.11
Threonine	0.02	0.04
Nutrient	Chemical composition	
Crude Protein	19.0	19.0
ME	2730	2630
Crude Fiber	5.738	6.70
Ether Extract	5.134	4.841
Ash	7.038	7.207
NFE****	52.788	52.174
Moisture	10.302	10.078

*Positive Control (1), **Negative Control (2), ***Vitamin Premix contained all the required minerals and vitamins, ****Nitrogen free extract.

diet plus 0.25 g/kg enzyme, group D diet was containing low energy diet plus 0.5 mg/kg enzyme, group E diet was having low energy diet plus 0.5% citric acid, group F was containing low energy diet plus 1.5% citric acid, group G diet was containing low energy diet plus 0.25 g/kg enzyme plus 0.5% citric acid and group H diet had low energy diet plus 0.5 g/kg enzyme plus 1.5% citric acid, as complete dietary treatments, ingredient and chemical composition of diets are shown in Table 1 and 2, respectively.

The feed intake and body weight was recorded to calculate FCR. At the end of the experiment (at 35th day) two birds from each replicate were selected randomly for blood collection to estimate blood glucose level. Blood was collected before feeding and after feeding at an interval of 1, 2 and 3 hours from wing vein and blood glucose was determined using blood chemistry Analyser (Germnay M-300). Nutrient composition of the diets samples was calculated by performing Weende analysis using AOAC (2000) methods and Metabolisable Energy (ME) of treatment diets was calculated using Carpenter and Clegg equation (Leeson and Summer 2005).

ME (kcal/kg) = 53 + 38 [(crude protein %) + (2.25 x ether extract %) + (1.1 x starch, %) + (sugar %)].

Data collected was subjected to ANOVA for a completely randomized design (Steel et al 1997). Mean differences were calculated by using Duncan's multiple range test. P<0.05 levels was accepted statistically significance.

Results

The weight results at the age of 35th day (Table 3) showed that broilers of basal diet (group A) attained maximum weight gain which was followed by low energy diet having 0.5

Table 3. Average body weight, feed consumption and feed conversion ratio of broilers in different experimental groups (0-35 day).

Treatment groups	Average Body Weight (g)	Feed Intake (g)	FCR
A	1727.80 ± 6.50^{a}	3236.60 ± 6.77^{c}	1.87 ± 0.01^{e}
B	1691.20 ± 5.81^{de}	3260.00 ± 7.91^{a}	1.93 ± 0.00^{a}
C	1698.60 ± 5.03^{cd}	3245.00 ± 5.15^{b}	1.91 ± 0.00^{bc}
D	1723.20 ± 6.06^{a}	3235.00 ± 6.82^{cd}	1.88 ± 0.01^{de}
E	1685.00 ± 6.08^{e}	3227.00 ± 5.83^{de}	1.92 ± 0.01^{b}
F	1683.40 ± 5.98^{e}	3215.20 ± 7.29^{f}	1.91 ± 0.01^{bc}
G	1701.80 ± 6.30^{bc}	3235.80 ± 4.44^{c}	1.90 ± 0.01^{c}
H	1707.40 ± 7.47^{b}	3218.80 ± 6.18^{ef}	1.89 ± 0.01^{d}

a, b, c, d, e, f: Values with different superscripts in a column differ significantly (P<0.05).

Table 4. Metaboliseable energy (Mcal /kg) of different experimental groups.

Treatment groups	ME
A	2.72 ± 0.02^{a}
B	2.64 ± 0.08^{abc}
C	2.67 ± 0.07^{abc}
D	2.72 ± 0.10^{a}
E	2.60 ± 0.03^{bc}
F	2.59 ± 0.07^{c}
G	2.72 ± 0.07^{a}
H	2.70 ± 0.10^{ab}

a, b, c, : Values with different superscripts in a column differ significantly (P<0.05).

Table 5. Glucose (mg/dl) concentration in blood of different experimental groups.

Treatment groups	Before feeding	1 hr after	2 hr after	3 hr after
A	160.20±4.82^{d}	232.60±7.27^{c}	240.14±7.55^{c}	235.08±7.09^{d}
B	153.00±7.38^{de}	223.80±6.46^{d}	237.36±2.66^{c}	224.64±5.08^{e}
C	194.00±7.21^{c}	240.00±5.39^{bc}	260.40±5.32^{b}	244.48±7.08^{c}
D	210.00±5.15^{b}	264.60±6.43^{a}	286.56±4.92^{a}	260.38±7.46^{b}
E	150.00±7.38^{e}	220.14±4.67^{d}	232.14±5.16^{c}	221.14±4.91^{e}
F	147.00±6.04^{e}	222.70±7.16^{d}	236.22±5.57^{c}	227.28±5.43^{de}
G	190.00±7.38^{c}	246.74±6.86^{b}	267.88±7.27^{b}	250.08±6.82^{c}
H	222.40±7.57^{a}	270.40±5.29^{a}	280.36±6.59^{a}	270.42±6.65^{a}

$^{a, b, c, d, e}$: Values with different superscripts in a column differ significantly (P<0.05).

g Zympex/kg feed, low energy diet having 0.5 g Zympex/kg feed plus 1.5% citric acid. The differences of average weights of various groups when compared with group A (control), revealed that the weights of group B, C, E, F, G and H were decreased (P<0.05) but there was no difference (P>0.05) between group A and D. While comparison of various groups with B (negative control) revealed that the weight of group A, D, G and H was higher (P<0.05) than B, but groups C, E and F showed no difference (P>0.05) in weight gain than B. It is evident from the above results that there is increase in weight of broilers of group D, showing positive effect of enzymes as compared to group B. During the study group B and C showed (Table 3) higher feed consumption (P<0.05) while group E, F and H showed lower feed intake (P>0.05) although group D and G showed no difference as compared to group A (control) while on comparison with negative control (group B) all groups showed reduced feed intake (P<0.05). The feed conversion ratio of test groups at different ages is shown in Table 3. All groups exhibited poor FCR (P>0.05) except group D which showed better FCR (P>0.05) as compared to group A. While all groups showed better FCR (P>0.05) when compared with negative control group B. ME is lower (P>0.05) in group E and F as compared to group A while group D,G and H has ME numerically equal to control group A (Table 4). There was no difference (P<0.05) in ME level in all groups when compared with negative control group B. M.E in groups D, G and H is equal to control group A which shows the action of enzyme or acid which results in more release of energy. Glucose level is higher (P>0.05) in all groups except groups C when compared with group A and B after one hour of feeding (Table 5). Its level is also higher in all groups except E and F at 2 and 3 hour interval as compared to control group A and negative control B.

Discussion

The benefits of enzyme supplementation in feed for improvement in utilization of nutrients and performance of bird are well known (Bedford and Morgan 1996). Some cereals grains like barley, wheat and corn may have non-starch polysaccharides which show their suppressive and anti-nutritional effect on the broiler performance and addition of enzyme can minimize the adverse effect (Annison and Choct 1991).

In some studies it has already been reported that the addition of multi-enzyme in feed has a positive effect on growth performance in broiler (Zanella et al 1999, Ghazi et al 2002, Yu et al 2007). However the enzyme efficiency is related with age of the bird also as older birds have more microbial population and more intestinal fermentation rate so they can combat with viscous feed ingredients (Vranjes and Wenk 1995, Choct et al 1996). In another study by Lazaro et al (2003) it was observed that by reducing viscosity of digesta enzyme efficiency may increase and results in better performance of broiler.

In this study supplementation of multi-enzyme has significantly negative effect (P<0.05) in different age groups as compared with the control group A except group D. But when low energy diets supplemented with different level of enzymes and acid was compared with control group B, it showed significant improved impact in group D, G and H which shows the activity of enzymes and acids as these groups have less energy and even group D performance was equal to group A although it was non-significant but it clearly showed that inspite of having less energy this diet performance was competing with normal diet group A which was the evidence of enzymes effect (Hajati 2010), and it was according to the finding of previous studies by Zanella et al (1999) who found that Avizyme (a cocktail enzyme) supplementation had resulted in improved weight gain. It was also observed in previous studies that NSPs digesting enzymes usually resulted in increased utilization of nutrients which leads to better weight gain, FCR and reduced digesta viscosity and decreased environmental pollution (Broz and Ward 2007, Costa et al 2008). When we compared groups D, G and

H with group B (control-2) it revealed significant positive weight gain (Table 3) in last two weeks which indicated the efficiency of exogenous enzyme. Similar findings were also reported by (Kavitha Rani et al 2003) that addition of multi-enzyme resulted in increased weight. Some studies (Gao et al 2007, Olukosi et al 2007, Yu et al 2007, Paul et al 2007, Gutierrez del Alamo et al 2008, Ao et al 2009) also reported the positive effect of enzyme supplementation on performance of broiler. The addition of organic acid resulted in low growth rate ($P<0.05$) in this study. While Sheikh Adil et al (2010) in their study on the supplementation of organic acids in broiler found that addition of lactic, fumaric and butyric acid in broiler diets resulted in better ($P<0.05$) growth performance and FCR. Similar findings were reported by Henry et al 1987 and Owens et al 2008. Organic acid are also known to reduce the pH of digesta which is favorable for exogenous enzyme activity (Dibner and Buttin 2002, Abdel-Fattah et al 2008). The results of the present study are also completely in line with the studies conducted by Cave (1984), Brown and Southern (1985), who found no positive effect of organic acids on broiler production parameters. In some studies it was also found that there was no significant effect of citric acid on growth and this is supported by the findings of Bolton and Dewar (1964) and Hume et al (1993). Some studies (Skinner et al 1991, Paul et al 2007, Abdel-Fattah et al 2008) reported that the growth rate was improved when it was supplemented with organic acids. This variation may be different levels of acid used or type of the acid which different scientist used in their experiments. The controversy in the results might be because of use of acetic acid in low energy diet in this study. As it is previously mentioned that decreased effect of pH by organic acid is beneficial for the activity of exogenous enzymes but in this study it not obvious.

The previous studies showed the reduced feed intake (Table 3) by enzyme supplementation (Kocher et al 2002) and similar observations were also reported by Sikka and Chawla (2002). The difference might be due to low energy diet used in current study but inspite of having low energy feed consumption in group C, D and G was near the control group A but when compared with group B (negative control) it showed significant reduced feed intake in all groups which were having enzyme supplementation as previous studies also reported the similar results regarding feed consumption and weight gain (Gao et al 2007, Yu et al 2007 Gutierrez del Alamo et al 2008).

FCR (Table 3) was recorded significantly poor in all groups except group D and H as these had FCR equal to group A in spite of having low energy. When compared other groups with control group-2 (B) it showed better FCR, which was in line to the findings of Zanella et al (1999) who observed that supplementation of multi-enzyme results in better FCR. The similar findings were also observed in other studies that enzyme addition results in better feed efficiency (Kavitha Rani et al 2003, Singh and Khatta 2003, Wu et al 2004). The findings of the experiment are also in line with the findings of Abdel-Fattah et al (2008) who reported that birds given diets with combination of organic acids improved FCR and weight resulting in better economics. Ao et al (2009) found in their experiment by feeding broiler with enzymes and citric acid that citric in the absence of galactosidase enzyme reduced the feed to gain ratio.

The metabolisable energy (Table 4) was non- significant ($P>0.05$) in all groups when compared with group A and B which showed that low energy diets groups were also having same ME as control groups which was the evidence of enzyme activity in low energy diet. These results are in line with the studies of Schang et al (1997) and Graham et al (2002) who reported that increase in ME with addition of enzymes. The groups E and F which was only supplemented with different levels of citric acid are lowest in ME which was due to addition of acid in diet which was already deficient in energy. By supplementation of feed especially low energy diet with NSP enzyme can increase the apparent metabolisable energy which resulted in better growth performance. Organic acids are mostly used to kill pathogenic bacteria and suppress the broiler growth which is compensated by the enzyme.

Glucose level (Table 5) is higher ($P<0.05$) in all groups except in E and F as compared to control group A and also it is higher in all groups except C, E and F when compared with control group B. The results are in agreement with the findings of Ao et al (2009) who reported the increase in sugar contents with addition of enzyme and acid. Balamurugan and Chandrasekaran (2010) also reported increase ($P<0.05$) in blood glucose level in broiler fed diets supplemented with enzymes. While Sheikh Adil et al (2010) reported no effect of organic acid alone on glucose level in broiler birds. Hernandez et al (2006) also reported that organic acid supplementation in broiler diets has no significant on blood metabolites. But it may be considered that organic acid in combination may have improved effect on glucose level because of the pH reduction which enhances the activity of enzymes.

Conclusion

Supplementation of low caloric diet with exogenous multi-enzymes resulted in better performance and also it was competing with the performance of basal diet group which was evidence of enzyme activity. While citric acid addition has shown no pronounced effects when added in low caloric diet.

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