T114 Broiler chickens fed anticoccidial drug feeds supplemented with AGPs and or Actigen®, a specific traceable carbohydrate fraction derived from the cell wall of yeast. Greg Mathis1, Brett Lumpkins2, James Pierce2, Ted Selfon2, Adam Burnsed2, 1Southern Poultry Research, Inc., Athens, GA, 2Alttech, Inc., Nicholasville, KY

A 52 day broiler study was conducted to compare the performance of commercial broilers fed Actigen®, a specific traceable carbohydrate fraction derived from the cell wall of yeast, versus a traditional AGP program of Bacitracin Methylene Disalicylate (BMD) and Virginiamycin (VIR). The treatments were T1 no additive (control), T2 Actigen™ 400 g/t (D0-52), T3 BMD 50 g/t (D0-31), VIR 20 g/t (D31-52), and T4 (T2 + T3). Starter feed was fed from D0-17, grower D17-31, and finisher D31-52. The treatments were replicated in twelve blocks, randomized within blocks of four pens each. Fifty commercial strain (Cobb X Cobb) male broiler chickens were placed into each pen. Floor space was 0.77 sq. ft. per bird. Birds were raised on built up litter. Salinomycin (60 g/t) + 3-Nitro 20 (45.4 g/t) were added to all starter and grower feeds. Live bird weights and feed consumptions were measured on D17, 31, 42, and 52. The inclusion of Actigen™, AGP program, and the combination program significantly (p<0.05) improved FCR at each weigh period compared to T1 (control). At D52 the combination T4 program average FCR (1.777) was significantly better than either single program T2 (1.820) or T3 (1.807) or T1 (1.872) FCRs. On D31, 42, and 52, the average bird weight gain for all supplement programs T2 (2.865 kg), T3 (2.900 kg), and T4 (2.928 kg) were significantly improved compared to T1. At each weigh period, there was no significant difference in average bird weight gain between the Actigen™ and AGP fed birds. Thus, both Actigen™ and AGPs improved performance of broiler chickens fed an anticoccidial program, with the greatest improvement coming from the birds fed the combination of Actigen™ and AGPs.

Key Words: Actigen, BMD, Virginiamycin, Salinomycin, Anticoccidial

T115 Evaluation of a multispecies probiotic for the control of Campylobacter in poultry Michaela Mohri1, Mar Bärmáes2, Gerd Schatzmayr1, BIOMIN Holding GmbH, Herzogenburg, Lower Austria, Austria, 1CESAC – Centre de Santal Avicola de Catalunya i Aragó, Reus, Catalonia, Spain, 1BIOMIN Research Center, Tuiln, Lower Austria, Austria

The use of specific probiotics has been shown to be an effective means for controlling pathogens in poultry flocks. Potential probiotic candidates were isolated from the gastro-intestinal tract of chickens and tested in vitro with a co-cultivation agar plate assay for the ability to inhibit the growth of Campylobacter jejuni spp. jejuni. The most promising strains with inhibition indexes ranging from 1.27 to 1.50 were evaluated for further important probiotic criteria. Based on these results a multispecies probiotic product consisting of strains belonging to the genera Enterococcus, Pediococcus, Lactobacillus and Bifidobacterium was designed (PoultryStar®, BIOMIN GmbH). The efficacy of the product to reduce C. jejuni in experimentally infected broiler chicks was evaluated in a series of experiments. In one in vivo experiment where all chicks were orally challenged with C. jejuni it was shown that the application of 2 mg/bird/day of the multispecies probiotic via the drinking water resulted in a significant reduction (P<0.05) of the cecal colonization of C. jejuni. In another in vivo experiment 72 day old broiler chicks were randomly assigned to three treatment groups, a positive control group, a trial group which received 2 mg/bird/day and a trial group which received 20 mg/bird/day of the multispecies probiotic product via the drinking water. The chicks were challenged with C. jejuni on day one by introducing 4 seeder birds per group which were orally inoculated. At day 8 and 15 the birds in the probiotic group obtained Campylobacter counts in the cecal content of less than 2 log10 cfu/g whereas the mean log counts in the positive control group were with 7.81 log10 cfu/g at day 8 and 7.85 log10 cfu/g at day 15 significantly higher (P<0.05). In conclusion the data of these studies suggest that the multispecies probiotic caused a significant reduction in cecal colonization of C. jejuni and might be beneficial for the control of Campylobacter in poultry flocks.

Key Words: multispecies, probiotic, Campylobacter jejuni, food-borne illness

Nutrition V

T116 Evaluation of Poultrygrow 250™ (a protease) supplemented in a broiler diet with decreased levels of Digestible Lysine and Crude Protein from 0 to 42 days of age. Brett Lumpkins1, Greg Mathis1, Robert Gauthier2, 1Southern Poultry Research, Inc., Athens, GA, 2Jefo, Saint-Hyacinthe, Québec, Canada

In today’s rising economic climate the ability to formulate diets has become more difficult with the increased cost of feed ingredients. Feed enzymes have become a focal point for nutritionists as a means of helping to lower feed cost. The purpose of this study was to determine the effect of supplementing Poultrygrow 250™ (a protease) in a broiler diet with two decreased levels of digestible lysine and crude protein. A 42 day broiler floor-plan study was conducted feeding the following treatments in a three phase feeding program: T1) commercial grade corn soybean meal diet (1.21, 0.96, and 0.81% digestible lysine, 22.50, 20.00, and 17.25% crude protein in the starter, grower, and withdrawal diets, respectively), T2) same as T1 with a decrease of digestible lysine and crude protein by 5%, T3) same as T2 plus protease, T4) same as T1 with a decrease of digestible lysine and crude protein by 8%, and T5) same as T4 plus protease. All feeds were pelleted. A randomized block design with 8 replications of 50 birds per pen was used. Feed and water were available ad libitum. Bird weights and feed consumption (kg) were recorded by pen at the initiation of the study, 19, 35 and 42 d of age. Weight gain, feed intake, FCR, were calculated and the data were analyzed statistically by Tukey’s mean separation test. At 19, 35 and 42 d, birds fed T2 and T4 had a significant (P<0.05) depression in performance (FCR and BWG) compared to the birds on the commercial diet (T1). At 19 d when the birds were fed the diets with Poultrygrow 250™ (T3 and T5) there was a significant improvement in FCR by 1.7 and 1.8%, respectively, bringing the values similar to those used in standard commercial grade diets, based on performance when fed from 0 to 42 d of age.

Key Words: Digestible Lysine, Crude Protein, Protease, Performance, Broilers

T117 Efficacy of a modified 6-phytase on apparent P and Ca digestibility in laying hens Imke Kühn1, Klaus Männér2, Mike R. Bedford3, 1AB Vista, Darmstadt, Hessen, Germany, 2Institut of Animal Nutrition, Free University Berlin, Berlin, Germany, 3AB Vista, Marlborough, UK

The efficacy of different application rates of a thermotolerant 6-phytase (QPT2) was studied in laying hens fed phosphorus reduced diets.
Two broiler chicken trials were conducted to evaluate the efficacy of NSP degrading enzymes in wheat-based diets with lowered (uplift) or normal (on top) ME concentrations. In Exp 1, 3200 newly hatched chicks (40/pen) were used in six dietary treatments, with 10 replicates each. In a Normal ME basal diet, the following enzymes were added: a commercial product A (A, xylanase/beta glucanase, 2kg/tonne), Product R (0.5kg/tonne), Product B (xylanase, 0.1kg/tonne), XB1 (low at 0.25kg/tone, or high at 0.35kg/tonne), or XB2 (low at 0.25kg/tonne, or high at 0.35kg/tonne). Both trials lasted for 41 days, with growth performance and health status monitored during the Starter, Grower, and Finisher periods. The results from Exp 1 showed that a negative effect on dietary ME (-120 kcal/kg) negatively impacted the FCR of the birds (P < 0.0001). But this negative effect was offset by all three enzyme blends with various xylanase/beta glucanase activities (XB1, XB2, or XB3, 0.25kg/tonne) in the starter and grower diet. In conclusion, a NSP enzyme could be added directly in a normal ME diet to further optimize the feed efficiency of broiler chickens.

Key Words: microbial phytase, broiler, growth performance, phytase matrix

T110 Impact of exogenous non-starch carbohydrates and phytase on nutrient digestibility, gut health and integrity in broilers

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The study was conducted to determine the effects of supplementing poultry diets with exogenous enzymes (Rovabio® Max; 28814 and 15792 U/g xylanase and phytase activities respectively) on performance, nutrient digestion and absorption, gut health and integrity, and immune status. A total of 445-day-old broilers in 8 replicate pens were randomly assigned to 4 treatments made up of a positive control (PC), a negative control (NC) diet which was lower than the PC diet by 100 FTU/kg phytase in the starter diet and 250 FTU/kg phytase in the grower diet. From d 0 to 16, feed intake (FI) was reduced (P ≤ 0.05) in broilers fed NC 2 or NC 3 compared to broilers fed the PC. Body weight gain (BWG) and feed efficiency (FCR) were reduced (≤ 0.05) in broilers fed the NC diets compared to broilers fed the PC. From d 0 to 16, FCR was improved (P ≤ 0.05) in broilers fed NC 2 or NC 3 compared to the PC. Phytase supplementation to the NC diets increased (P ≤ 0.05) FI and improved (P ≤ 0.05) BWG comparable to the PC. From d 0 to 16, FCR was improved (P ≤ 0.05) in broilers fed NC 2 or NC 3 compared to the PC. From d 0 to 16, FCR was improved (P ≤ 0.05) in broilers fed NC 2 or NC 3 compared to the PC. Phytase supplementation to the NC diets increased (P ≤ 0.05) FI and improved (P ≤ 0.05) BWG comparable to the PC. In conclusion, a novel microbial phytase supplemented at 250 or 500 FTU/kg improved broiler growth performance in low Ca, P, Na, ME, and amino acid diets. The growth response to phytase supplementation was larger in the starter (d 0 to 16) phase than the grower (d 16 to 34) phase.

Key Words: microbial phytase, broiler, growth performance, phytase matrix

T118 Efficacy of non-starch polysaccharide (NSP) degrading enzymes in wheat-based broiler chicken diets with or without metabolizable energy uplift

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Two broiler chicken trials were conducted to evaluate the efficacy of NSP degrading enzymes in wheat-based diets with lowered (uplift) or normal (on top) ME concentrations. In Exp 1, 3200 newly hatched chicks (40/pen) were used in six dietary treatments, with 10 replicates each. In a Normal ME basal diet, the following enzymes were added: a commercial product A (A, xylanase/beta glucanase, 2kg/tonne), Product R (0.5kg/tonne), Product B (xylanase, 0.1kg/tonne), XB1 (low at 0.25kg/tone, or high at 0.35kg/tonne), or XB2 (low at 0.25kg/tonne, or high at 0.35kg/tonne). Both trials lasted for 41 days, with growth performance and health status monitored during the Starter, Grower, and Finisher periods. The results from Exp 1 showed that a negative effect on dietary ME (-120 kcal/kg) negatively impacted the FCR of the birds (P < 0.0001). But this negative effect was offset by all three customer enzymes, resulting in similar FCR to that of the Normal ME diet (P > 0.05). Product R also improved FCR but failed to produce the same result as by the Normal ME diet (P < 0.05). In Exp 2, adding the enzyme on top of the normal ME diets showed a strong tendency to further improve FCR (P = 0.050). From Days 0-41, Product A, Product R, Product B, XB2 Low, XB2 High, XB3 Low and XB3 High improved FCR by 1.5, 1.7, 1.4, 0.8, 1.5, 1.5, and 1.7% respectively (P = 0.050).

In both trials, the dietary treatments did not influence the body weight, feed intake, or mortality (P > 0.05). In conclusion, a NSP enzyme could improve growth performance effectively in a ME reduced diet, allowing cost saving due to less fat addition (up to 2%). Alternatively, a NSP enzyme could be added directly in a normal ME diet to further optimize the feed efficiency of broiler chickens.

Key Words: phytase, ileal digestibility, layer, phosphorus

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kcal ME/kg, 0.5% pt CP/AA, 0.15% avP and 0.1% Ca) and 2 NC diets supplemented with Rovabio® Max at 0.05 and 0.075g/kg diet. On days 21 and 42, one bird per pen was killed for gene expression analyses of mucin (MUC2), interleukin 1 (IL-1β) and phosphorus transporter (NaPiIIb) by real time polymerase chain reaction in mucosal samples from the duodenum, jejunum, ileum, and 5 birds per pen were moved to cages for digestibility study. For performance response criteria, body weight (BW) and average daily gain (ADG) were linearly increased (P < 0.05) by enzyme supplementation both on days 21 and 42. Enzyme linearly increased (P < 0.05) feed intake (FI) from day 21 to 42 and overall. The gain to feed ratio was linearly improved by multi-enzyme complex from day 0-21, and quadratically from day 21 to 42 and overall (P < 0.05). Additionally, enzyme addition led (P < 0.05) to higher serum phosphorus (P) concentration. Analysis of gene expression changes did not reveal significant differences between treatments. Furthermore, although there was no significant differences in dry matter (DM), energy and nitrogen (N) apparent ileal digestibility (AID) on day 21, AID of P was linearly increased (P < 0.05) by enzyme addition. Additionally, on day 42 exogenous enzymes linearly increased (P < 0.05) the AID of DM, energy, P and N. Overall, these results confirmed that Rovabio® Max can improve growth performance, energy and P utilization of broiler chickens fed diets with reduced levels of ME, CP/AA, avP and Ca.

Key Words: Broiler chicken, Rovabio® Max, Nutrient Digestibility, Immune response, Gut health

T121 Effects of a multi-species probiotic to control lameness in broiler chickens using the wire flooring model Amanda Jordan1, Robert F. Wideman1, Igal Pevzner1, 1Biomin USA, San Antonio, TX; 2University of Arkansas Department of Poultry Science, Fayetteville, AR; 3Cobb-Vantress Inc., Siloam Springs, AR

Bacterial chondronecrosis with osteomyelitis (BCO) is the most common cause of lameness in commercial broilers. With the concerns of BCO on the rise our hypothesis of the present experiments was to specifically measure the efficacy of probiotic administration against BCO in broiler chickens. In four independent experiments the usage of a multi-species probiotic product (PoultryStar®, BIOMIN GmbH) on a preventative scale was investigated starting at day one of age against lameness and the effects caused by lameness. Broiler chicks from three different commercial hatcheries were placed at >60 per pen at day one of age. Feed and water were given ad libitum. In experiments 1 to 4, the experimental diet consisted of control feed mixed with the multi-species probiotic product. Lame birds were removed as soon as the onset of lameness was observed, and were euthanized via CO2 gas inhalation. At day 14 of age the population was reduced to between 50 and 55 of the largest, healthiest chicks per pen. In three of the experiments survivors at day 56 were weighed, euthanized and necropsied to assess sub-clinical lesion incidences in the proximal heads of the femora and tibiae. In the four experiments using four commercial lines, broilers grown on wire flooring developed high levels of lameness attributable predominately to BCO. The pathogenesis of BCO is not instantaneous; accordingly many broilers that did not exhibit lameness nevertheless did possess early evidence of BCO through clinical lesion development. In the four independent experiments the results showed that by feeding the probiotic product the number of birds which developed signs of lameness could be significantly decreased (P≤ 0.05). These experiments indicate that the commercially available multi-species probiotic administered on a preventative basis could potentially show a reduction of lameness in broilers.

Key Words: probiotics, flooring, lameness, broilers, bacterial chondronecrosis/osteomyelitis

T122 The role of Peptide YY in the mode of action of dietary xylanase Helen Masey O’Neill1, M. R. Bedford1, Sudipto Halder2, AB Vista Feed Ingredients, Marlborough, Wiltshire, United Kingdom (Great Britain), 2West Bengal University of Animal & Fishery Sciences, Kshudiram Bose Sarani, Kolkata, India

It has long been established that the mode of action of dietary xylanase in viscous cereal based diets is via a viscosity reduction leading to increased nutrient digestibility. However, it is becoming increasingly clear that xylanase is also efficacious in corn-based diets where it has been shown to improve FCR, not by stimulating FI but by increased BWG, and therefore presumably by an increase in feed use efficiency. Xylanase is known to produce oligomeric unabsorbed polysaccharides which are rapidly fermented in the caeca. The products of fermentation have been seen in mammalian species to stimulate the production of various gut hormones which influence gut function in the upper regions of the tract. Increased gastric retention is one such effect and this may be implicated in improved digestibility of food material. Peptide hormone YY, released from the L cells of the small intestine and colon, is one example. Two hundred and eighty-eight, day old straight run Cobb broilers were penned, 12 birds per pen with 6 replicates per treatment between day 1 and 42. Enzyme doses of 0 or 16000U/kg and two different diets (basal and basal – 100kCal; both based on corn/soy but differing in fat content), were used in a 2 x 2 factorial design and subsequent ANOVA. Significance was accepted at a probability equal to or less than 0.05. Plasma PYY was measured at day 42 using an ELISA assay. Feed intake, body weight gain and FCR were also measured. There was a significant interaction between diet and enzyme dose (P=0.016) on plasma PYY. That is, the size of the effect of enzyme dose was dependent on the diet type. However, the pattern of dose response was the same for both diets and both main effects of diet and enzyme dose were significant (P=0.001 in both cases) with the 16000U/kg dose resulting in significantly more PYY than the 0 dose, for both diets. Feed Conversion Ratio was significantly better with the basal diet (P=0.035). However, there was no significant effect of enzyme dose (P=0.689) or interaction between enzyme dose and diet type (P=0.852). There were no significant effects of FI. Body Weight Gain was better with the basal diet (P=0.035) but there was no significant effect of enzyme dose (P=0.876) or interaction between enzyme dose and diet type (P=0.562). Peptide YY appears to be released by the gut of broilers in response to a 16000U/kg of xylanase and therefore may be implicated in the mechanism of action. Further experiments are required to investigate whether, in broilers, this hormone impacts upon gut characteristics, digestibility and subsequent performance.

Key Words: Broiler, Enzyme, Hormone, PYY, Xylanase

T123 Development of RONOZYM® ProAct - a unique protease for broiler feed Katrine Pontoppidan1, Vibe Glitsoe1, Dan Pettersson1, Nelson Ward2, Roland Brugger2, 1Novozymes A/S, Bagsvaerd, Denmark, 2DSM Nutritional Products Inc., Parsippany, NJ

The purpose of the extensive research project summarized here was to identify a protease that can improve amino acid digestibility and animal performance. Based on the belief that stability and activity in the gastro-intestinal tract are of equal importance for feed enzymes, the development process focused on stability and ability of proteases to degrade feed protein under conditions relevant for the gastro-intestinal tract. Acid stability was evaluated as the residual protease activity at pH 7 (40°C) using a synthetic substrate after incubation at pH 3 for 2 hours (40°C). Protease efficacy during digestion was evaluated using an in vitro model simulating digestion of a corn-soy diet in the stomach (pH 3 with pepsin, 1 h, 40°C) and small intestine (pH 7 with pancreatic enzymes, 4 h, 40°C) and subsequent analysis of the degree of protein hydrolysis (DH) using a colorimetric assay.
In the screening of a large number of experimental proteases, a serine protease from the strain *Nocardioopsis* was shown to be the best candidate with respect to the parameters described above. In addition, the *Nocardioopsis* protease also demonstrated significantly better acid stability and activity in the gastro-intestinal digestion model (P<0.05) when compared to different commercial protease containing products.

Based on these findings the *Nocardioopsis* protease was selected as the best protease to survive the passage through the acidic conditions of the stomach and act in the small intestine, and was therefore developed into the product, which today is marketed as RONOZYME® ProAct (DSM Nutritional Products).

Key Words: Enzymes, Protease, Stability, In vitro digestion, Protein

**T124 Compatibility of RONOZYME® ProAct with phytases and xylanases** Katrine Pontoppidan1, Vibe Glitsoe1, Dan Pettersson1, Nel-xylanases

RONOZYME® ProAct was added to the incubations compared to when the enzyme alone or in combination with RONOZYME® ProAct (DSM Nutritional Products) does not negatively influence the efficacy of different commercially available phytases and xylanases.

In conclusion, RONOZYME® ProAct significantly increased DH (P<0.05).

Key Words: Enzymes, Phytase, Phytase, Phytase, Xylanase, In vitro digestion

**T125 Effect of Rovabio and Hemicell Enzymes on Commercial Turkey Hen Performance** J L Grimes1, C Stark1, D McIntyre2, E Guaiume3, J E Nixon1, I B Baraschi1, **North Carolina State University, Raleigh, NC**, J E Dixon, Alpharetta, 1Cargill Meat Solutions, Witchita, KS

Feed costs are the major cost factor for rearing turkeys. Feed enzymes have the potential to lower feed cost and improve turkey performance. Commercial enzymes (E) need to be tested and compared to assess their value to the commercial turkey industry. In this trial, we tested two commercial feed enzymes (Rovabio and Hemicell) for feeding to commercial turkey hens. There were four feed treatments (T) using Nicholas LW hens. The birds were fed a series of commercial diets. The treatments were randomized within 2 blocks of 12 pens (6 pens per treatment, 24 pens total). There were 40 hen poults placed in each pen (94 ft²/pen.). The treatments were T1 - Positive Control (PC); T2 - Negative Control (NC), T3 minus 90 kcal/kg; T3 - Rovabio, {T2 plus Rovabio}; and T4 - Hemicell, {T2 plus Hemicell}. The birds were weighed by pen at placement and then individually at 6, 10 and 15 wk. A pelleted basal feed (BF) was provided by a commercial feed mill and then processed at the NCSU feed mill. The BF was augmented to make the 4 different treatments. Extra fat was applied to make the PC (90 kcal/ kg above T2, T3, & T4). The E was applied as a liquid by use of a hand-held sprayer. Data were analyzed using GLM (SAS) with means separated using LS means. There was no effect (P>0.05) of T on BW. Feed conversions (FC) were also not different (P>0.05) due to T at 6 (1.36 ± 0.01) or 10 (1.76 ± 0.01) or from 6 to 10 (2.09 ± 0.02) or 10 to 15 (2.88 ± 0.04) wk. Birds fed PC diet had a (P=0.01) lower 15 wk FC than birds fed the NC diet (2.20 vs. 2.28 ± 0.02). The 15 wk FC for the birds fed E (T3, T4) were intermediate (2.24±0.02). Birds fed differing dietary energy levels with dietary nutrients held constant will have different feed conversions. Dietary E can restore FC lost by removal of dietary energy (fat). The degree of that restoration is probably dependent on many factors including E, rate of E inclusion, diet components, species of animal fed, and health and age of the animal.

Key Words: turkey, feed, enzyme, feed conversion, energy

**T126 Use of xylanase in broiler diets based in corn and soybean meal with different energy levels** Anelcir Scher1, Alexandre Pires Rosa1, Roberto Dornelles2, Lenise Schröder Boemo1, Rodrigo Bronzatti1, Daniele Pozzebon Rosa1, Camila Borba Santos1, 1Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil, 2Huvepharma, Santa Maria, Rio Grande do Sul, Brazil

The objective of this study was to evaluate the addition of HOSTAZYM X® in diets with different levels of energy based on corn and soybean meal for broilers. This study was carried out at Poultry Laboratory – LAVIC at The Federal University of Santa Maria – Brazil. Were used 2240 day old Cobb 500 male Broiler chicks. It was used a CRD factorial arrangement 4x2 (zero, 1%, 2% and 3% of reduction of metabolizable energy, with or without enzyme addition), totaling eight treatments with 7 replicates of 40 chicks each. Diets were mashed and had the same nutrient levels in the eight treatments at each phase, with the exception of the energy levels. Metabolizable energy levels in the diets without reduction were: 3050; 3100 and 3150 Kcal/Kg in the initial, growing and final phase respectively. Water and food were provided *ad libitum*. The enzyme was added at a dose of 500g/ton and had an activity of 3200 EPU/g. The data were submitted to ANOVA and Tukey test. At 42d, broilers from the group fed diets without energy reduction weighed 2661.5g and the groups submitted to diets with energy reduction of 2 and 3% weighed 2616.6 and 2591.3g, respectively (P<0.0001). The addition of the evaluated product increased body weight from 2616.3 to 2640.5g (P=0.0131). The reduction of energy level in 3% resulted in a lower feed intake at 42d and the addition of the enzyme increased feed intake from 4411.1 to 4447.0g in the 1-42d period (P=0.0899). It was observed interaction between the energy levels and the addiction of enzyme. From 22 to 35d, and 1 to 42d broilers fed diets with 2 and 3% energy reduction, with the addition of enzyme exhibited higher feed intake than those who fed diets with 3% of energy reduction without the addition of enzyme. The reduction of energy levels and the addiction of enzyme had no effects on feed conversion ratio. The addiction of HOSTAZYM X® promoted increases in body weight, feed intake and productive efficiency index.

Key Words: enzyme, performance, energy level