

## PRACTICAL ASPECTS OF ENZYME-PROBIOTICS PREPARATION SUPPLEMENTED TO QUAIL DIETS

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### ABSTRACT:

The aim of the current investigation is to demonstrate the effect of supplementing corn – soybean based diets with enzymes and probiotics preparation on quails performance. Four hundred and eighty one-day-old Japanese quail were distributed randomly to four equal experimental groups of 120 birds each. A conventional corn–soybean–meal based diet was used. The basal diet was supplemented with three experimental feed additives. The first feed additive was Xylam ( $\beta$ -xylanase, and  $\alpha$  amylase), it was added at the level of 0.5 g/kg diet. The second feed additive was Samu Biogen (*Bacillus subtilis* Matta, cellulase, protease,  $\alpha$ - amylase, and  $\beta$ -amylase), it was added at the level of 0.2 g/kg diet. The third feed additive was Organic Green Culture (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Apergillus oryzae*, protease, amylase, lipase, cellulase, and phytase); it was added at the level of 1.5 g/ kg diet. The results showed that Organic Green Culture supplementation increased final body weight by 13.4 while in case of Samu Biogen or Xylam increased by 8.5 and 7.1 respectively, compared with the control. Feed consumption, feed conversion ratio and mortality rate were also improved by the treatments. Organic Green Culture supplemented group had significantly higher weight of heart, thymus, spleen, and bursa compared with the control. The red blood cells (RBC's) count was significantly increased in response to Organic Green Culture followed by Samu Biogen then Xylam. The same trend was evident for white blood cells (WBC's) count. Plasma total protein, albumin and globulin were significantly increased in birds received Organic Green Culture and Samu Biogen compared to the control. Plasma aspartate aminotransferase (AST) and alanin aminotransferase (ALT) were not markedly affected by the feed additives used. Hemagglutination Inhibition titer test (HI) was significantly higher in birds received Organic Green Culture followed by birds receive Samu Biogen then birds received Xylam. The lowest bacterial count was observed with Organic Green Culture followed by Samu Biogen then Xylam supplemented group compared with the control. The same pattern was noticed with *E. coli* count. In conclusion, it is suggested that enzymes with probiotics preparations are suitable for mixing with corn- soybean based diets to improve quails performance and immunity.

**Key words:** Enzymes, probiotics, quail, performance and immunity.

### INTRODUCTION

The use of enzyme and probiotic products as poultry feed supplements has attracted considerable attention by feed manufactures and poultry producers as a means of improving poultry performance. Enzymes have been used to degrade different structural carbohydrates found in cereals, particularly those that are not digested by avian and mammalian enzymes, and those that are highly viscous and have high water-binding capacity. The beneficial

effects of enzyme supplementation are well documented (Sinlae and Choct, 2000 Bhat and Hazlewood, 2001, Bedford and Apajalahti, 2002, Yonemochi *et al.*, 2003, Lazaro *et al.*, 2004, Cowieson, 2005, Choct, 2006, Olukosi *et al.*, 2007, and Troche *et al.*, 2007). The enzymes have the advantage of a high specificity and the ability to operate efficiently at moderate temperature and pressure. The feed enzyme industry come into existence and it has gone through several phases of development. The first phase was the use of enzymes to enhance nutrient digestibility, focusing primarily on removing the anti-nutritive effects of non-starch polysaccharides (NSP) from diets based on viscous grains. Water-soluble  $\beta$ -glucans and arabinoxylans are the non starch polysaccharides (NSP) of major concern when being fed in poultry diets with high cereal grains content.  $\beta$ -Glucans are linear polymers of glucose with  $\beta$ -(1.3)(1.4) glycosidic links while arabinoxylans consist of long backbone chains of  $\beta$ -(1.4) anhydra- D-xylopyranosyl to which are attached single  $\alpha$ -L- arabinofuranosyl residues at the 2- or 3- position (Fincher and Stone, 1986). Enzymes allow the use of a wide range of ingredients without compromising bird performance and hence provide great flexibility in least-cost feed formulation (Ghazalah *et al.*, 2005). Hong *et al.* (2002) showed that enzyme supplementation proved benefits in corn-soybean diets for ducks. Addition of enzymes lowers digesta viscosity (Bedford and Classen, 1992) and promotes feed passage rate (Salih *et al.*, 1991). The effectiveness of enzyme preparations are not only on the basis of growth improvement and the decrease of the feed / gain ratio (Olukosi *et al.*, 2007) but may also decline the number of dirty chicks as well as lower water consumption and leads in turn to better sanitary and reduction of production risk. Thus, enzymes influence the conditions in the gut and consequently the composition and activity of intestinal microflora (Preston *et al.*, 2001).

A microbial involvement in the depression of nutrient retention in broiler has been indicated from observations that bird's performance can be improved with probiotics (Lee *et al.*, 2007). Some efforts are underway to establish whether selective culture of *L. acidophilus* would exert inhibitory action on *salmonella* as well as control the enteric pathogens (Caldwell *et al.*, 2004), and increased the birds resistance to *Eimeria acervulina* (Dalloul *et al.*, 2003). It has been reported that immunological and nonimmunological defenses play a role at the intestinal mucosal surface during *Eimeria* invasion (Lillehoj and Trout, 1993). Dalloul *et al.* (2003) showed that feeding probiotics may modulate the mucosal immune system enhancing the host's resistance to enteric pathogens. Properly selected cultures of probiotics, such as *lactobacillus* species, can overcome those lactobacilli found in the natural flora of the birds and exert inhibitory action toward salmonella in the intestinal tract of chickens (Stern *et al.*, 2001). Probiotics can increase nutrient utilization by providing enzymes in the gut capable of converting certain components of the diet into more digestible nutrients for the host animal. The specific function of probiotics may differ depending on the host animal and more importantly, on the characteristics of the probiotics.

Because it is thought that feedstuffs like corn and soybean that induce less viscosity may not benefit from the exogenous enzymes supplementation, thus the present study aimed to evaluate the effect of enzymes – probiotics supplementation to the corn-soybean based diet on quails performance. Moreover, to demonstrate to what extent the enzymes and probiotics could synergistically act to improve quail productivity.

**MATERIALS AND METHODS****Experimental Procedure:**

Four hundred and eighty one-day-old Japanese quail (*Coturnix Coturnix Japonica*) were obtained at day of hatch from a commercial hatchery, wing banded, individually weighed and were assigned randomly to four equal experimental groups of 120 birds each. Birds of each treatment were further subdivided into three replicates of 40 birds each. The birds were brooded in thermostatically controlled batteries. The lighting pattern was 23 h light: 1 h dark. Feed and water were added *ad libitum*. All birds were kept under the same managerial hygienic and environmental conditions throughout the entire experimental period that lasted for 6 weeks during the summer time.

**Diets:**

A conventional corn – soybean meal based diet was formulated to meet the minimum National Research Council recommendations (NRC, 1994). The basal diet was supplemented with three experimental feed additives. The first feed additive was Xylam; it was used as a source of enzyme supplement (A product of *Bacillus subtilis*; each gram contains endo-1.4  $\beta$ -xylanase 1260U,  $\alpha$  amylase 8000 U and wheat flour as a carrier. Xylam was added at the level of 0.5g / kg diet. The second feed additive was Samu Biogen; it was used as a source of enzymes and probiotics; each kg contains *Bacillus subtilis* not less than  $1 \times 10^{11}$  unit, cellulase 20000 unit, protease 250000 unit,  $\alpha$ -amylase 400000 unit,  $\beta$ -amylase 20000 unit, and corn starch as a carrier. Samu Biogen was added at the level of 0.2 g/kg diet. The third feed additive was Organic Green Culture; it was used as a source of enzymes and probiotics, each kg contains *Saccharomyces cerevisiae*  $1 \times 10^{11}$  IU, *Lactobacillus acidophilus*  $1 \times 10^{11}$ , *Bacillus subtilis*  $1 \times 10^{11}$ , *Apergillus oryzae*  $1 \times 10^{11}$ , protease 250000 IU, amylase 25000 IU, lipase 15000, cellulase 15000, and phytase 15000. It was added at the level of 1.5 g/kg diet. All feed additives were recommended by the producers. The composition and chemical analysis of the basal experimental diet is illustrated in Table 1.

**Growth performance traits:**

Individual body weight, weight gain and feed consumption were recorded weekly. Feed conversion was calculated on a replicates basis. The general status of birds was noticed and dead birds were removed, weighed and kept for post mortem examination.

**Lymphoid and other organs weight:**

At the end of the experiment, three birds of each replicate around the average weight were chosen, weighed and slaughtered after fasting for 10 hours. After slaughtering and complete bleeding, the internal organs (intestine, gizzard, liver, heart, bursa, thymus, and spleen) were removed carefully and weighed.

**Hematological traits:**

At the end of the experiment, five birds from each replicate were slaughtered after fasting for 10 hours. Two ml of blood were obtained from each bird in a sterile centrifuge tube containing heparin (20 IU/ ml) for counting the blood cells. In addition, three ml of blood were also taken from each bird in sterile tubes to get serum for assessing Hemagglutination Inhibition titer test (HI). The count of red blood cells (RBC's), white blood cells (WBC's) and differential white blood cells were determined according to the procedures outlined by Schalm *et al.* (1975).

**Table 1. Composition and calculated analysis of the basal diet**

<b>Ingredients</b>	<b>(%)</b>
Yellow corn	55.70
Soybean meal (44%)	33.13
Corn gluten meal	7.85
Limestone	0.85
Di-Calcium phosphate	1.59
DL-Methionine	0.08
L-lysine	0.18
Salt (NaCl)	0.32
Vitamins and minerals premix *	0.30
<b>Total</b>	<b>100</b>
<b>Calculated analysis**</b>	
Crude protein (%)	24.23
Metabolizable Energy (kcal/kg)	2900
Ca, %	0.80
Available P, %	0.45
Ether extract, %	2.58
Crude fiber, %	3.65
Lysine, %	1.30
Methionine, %	0.50

\* Vitamin and minerals premix provide per kilogram of diet the following: V.A 10000 IU; V.D3 2000 ICU; V.E 15 IU; V.K3 3mg; riboflavin 10 mg; Ca pantothenate, 10 mg; niacin, 20 mg; choline chloride, 500 mg; V.B12 10 µg; V.B6 1.5 mg; thiamine, 2.2 mg; folic acid, 1mg, biotin , 50 µg; Mn 55 mg; Zn 50mg; Fe 30; Cu, 10mg; Se,1mg.

\*\* NRC, (1994).

#### **Plasma biochemical analysis:**

Plasma was obtained immediately by centrifugation of heparinized blood for 10 min. at 3000 rpm and frozen rapidly in ependorf tubes until the time of analysis. Plasma globulin was calculated as the difference between plasma total protein and albumin. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also measured. All plasma samples were analyzed by using specific diagnostic kits (Bio Merieux, France) as recommended by **Armstrong and Carr (1964)** and **Bogin and Keller (1987)**.

#### **Immuno response test:**

The birds were vaccinated at 7 day of age by Lentogenic NDV Hitchner β vaccine in lyophilized form with minimum titer of  $10^9$  EID<sub>50</sub> (Izo Vac, 1000, Izo Laboratories, Italy). The lentogenic lasota vaccinal strain of NDV that passed two times in the allantoic sac of 9 – 11 days old chick embryo was used to vaccinate the birds at 17 and 27 days of age. The birds were vaccinated by eye drop using a standard dropper (0.5 ml / drop); each bird received 2 drops, one in each eye. At 21, 35, and 42 days of the experiment, three ml of blood were taken from each bird in a sterile centrifuge tube. After centrifugation, serum was used immediately for assessing HI titer. The test was performed according to the standard procedure described by **Majiyabe and Hitchner (1977)**.

**Bacterial Counts:**

**Total bacterial count:**

The test was performed according to the standard procedure described by **Tellez *et al.*, (1993)**. One gram of the cecal contents was diluted in 99 ml of nutrient broth or sterile physiological saline in 250 ml Erylen Myer flasks. Then 1 ml of this mixture is re-suspended in 9 ml of the same dilution system in 15 ml sterile glass test tubes. Then carry on the 10 fold serial dilution. From each test tube, transfer 1 ml from each dilution and spread on one nutrient agar medium plate, trypticase soy agar or brain heart infusion agar plates. Plates are incubated for 24 hrs at 37C. Then colonies are counted by a hand glass magnifier. The total bacterial count is obtained by multiplying the number of the colonies in the last dilution plate (lowest) by the inverse log. 10 of this plate. Thus we can obtain the total bacterial count. Total bacterial count did not include beneficial lactobacillus spp.

**Total E.coli count:**

One gram of the cecal contents was diluted in 99 ml of nutrient broth or sterile physiological saline in 250 ml Erylen Myer flasks. Then 1 ml of this mixture is re-suspended in 9 ml of the same dilution system in 15 ml sterile glass test tubes. Then carry on the 10 fold serial dilution. From each test tube, transfer 1 ml from each dilution and spread on one MacConkey agar plate, one brilliant green agar plate or one SS agar plate. Plates are incubated for 24 hrs at 37C. Then colonies are counted by a hand glass magnifier. The total bacterial count is obtained by multiplying the number of the colonies in the last dilution plate (lowest) by the inverse log. 10 of this plate. Thus we can obtain the total bacterial count.

**Statistical analysis:**

All data were subjected to statistical analysis by one way analysis of variance according to SAS program (SAS, Institute, Inc., 1996). Significant differences among means were tested by the methods of **Duncan (1955)**.

**RESULTS**

**Performance traits:**

The effects of Xylam, Sam Biogen and Organic Green Culture supplementation on Japanese quail's performance are illustrated in Table 2. Initial body weight did not statistically differ among experimental groups. Statistical differences were noticed in final body weight between control and the experimental treated groups. At the end of the experiment, Organic Green Culture supplementation increased significantly final body weight by 13.4% compared with the control. Meanwhile, final body weight of quail receiving Samu Biogen or Xylam increased significantly ( $P \leq 0.01$ ) by 8.5 and 7.1%, respectively compared with the control. Body weight gain was also influenced significantly by all experimental diets. Organic Green Culture supplemented diet increased weight gain by 14.3% compared with the control while weight gains of birds received diets supplemented with Samu Biogen or Xylam were statistically equal; their values were increased by 9.3, and 7.8%, respectively compared with the control.

Feed consumption was affected significantly by Organic Green Culture, Samu Biogen, and Xylam compared with the control. Supplementing Organic Green Culture to diet significantly decreased ( $P \leq 0.05$ ) feed consumption by 13.4% followed by quails receiving Samu Biogen (3.8%). Meanwhile, quails receiving Xylam in their diet significantly consumed more feed (2.0 %) than those fed the control diet.

Feed conversion ratio was significantly improved where quails receiving diet supplemented with Organic Green Culture exhibited the best feed conversion ratio followed by quail fed diet supplemented with Samubiogen, and Xylam compared with the control.

Mortality rate decreased with Organic Green Culture, Samu Biogen and Xylam supplemented diets. The birds received Organic Green Culture showed the lower mortality rate compared to the other groups. Samu Biogen and Xylam supplemented diets were numerically equal but less than those fed the control diets. The mortality % of quails fed diet supplemented with Organic Green Culture suggests a tendency to reduce mortality rate in quails fed this supplement.

**Table (2): Performance traits of growing Japanese quails as affected by different experimental treatments.**

Treatments Traits	Control	Xylam	Samu Biogen	Organic Green Culture	Significance
Initial Wt. (g)	8.3± 0.35	8.2± 0.34	7.8±0.30	8.1± 0.30	NS
Final Wt. (g)	169.0± 6.4 <sup>c</sup>	181.0±.9 <sup>b</sup>	183.3±3.4 <sup>b</sup>	191.7±3.8 <sup>a</sup>	**
Gain (g)	160.7± 6.4 <sup>c</sup>	173.3± 4.8 <sup>b</sup>	175.6±3.3 <sup>b</sup>	183.6±4.0 <sup>a</sup>	*
Feed intake (g)	801.8±3.5 <sup>b</sup>	817.7± 3.7 <sup>a</sup>	771.4±2.4 <sup>c</sup>	694.2±2.3 <sup>d</sup>	*
Feed:gain ratio	4.99± 0.2 <sup>d</sup>	4.73± 0.21 <sup>c</sup>	4.39±0.12 <sup>b</sup>	3.78±0.11 <sup>a</sup>	*
Mortality rate %	13.3 <sup>c</sup>	8.3 <sup>b</sup>	8.3 <sup>b</sup>	6.7 <sup>a</sup>	**

Means within row for each item having different superscript differ significantly

NS = Not significant

\* P≤0.05

\*\* P≤0.01

#### Internal organs weights:

Lymphoid organs weight and other organs indices data as affected by Organic Green Culture, Samu Biogen, and Xylam supplementation are demonstrated in Table 3.

Organic Green Culture supplemented group had significantly higher weight of heart, thymus, spleen, and bursa compared with the control. Samu Biogen and Xylam supplemented groups had statistically equal effect on bursa, spleen, and heart. Thymus percentage showed another trend where Organic Green Culture and Samu Biogen were statistically equal. Organic Green Culture supplemented group induced lower intestine weight followed by birds fed Samu Biogen and Xylam compared with the control while the gizzard weight was not affected. The high liver weight values noticed in birds fed Organic Green Culture followed by Samu Biogen and Xylam.

#### Hematological Assessment:

The effects of Organic Green Culture, Samu Biogen, and Xylam on some hematological parameters are tabulated in Table 4.

Organic Green Culture, Samu Biogen, and Xylam supplementation had a significant ( $P \leq 0.05$ ) and dynamic impact on the hematological profile of quail. The RBC's count was significantly increased in response to Organic Green Culture; its value was 131.5% of the control. The RBC's count in birds fed diet supplemented with Samu Biogen was significantly increased to 119.6% of the control, while in case of Xylam supplemented diet, it was increased to 117.4% of the control.

**Table (3): Lymphoid and other organs weight of growing Japanese quails as affected by different experimental treatments.**

Treatments Traits	Control	Xylam	Samu Biogen	Organic Green Culture	Significance
Liver (g)	2.00±0.19 <sup>b</sup>	2.20±0.06 <sup>b</sup>	2.40±0.29 <sup>b</sup>	2.65±0.15 <sup>a</sup>	*
Heart (g)	0.66±0.04 <sup>b</sup>	0.75±0.37 <sup>b</sup>	0.80±0.07 <sup>b</sup>	1.07±0.10 <sup>a</sup>	**
Thymus	0.18±0.03 <sup>c</sup>	0.22±0.03 <sup>b</sup>	0.25±0.03 <sup>a</sup>	0.27±0.03 <sup>a</sup>	*
Spleen (g)	0.05±0.01 <sup>c</sup>	0.09±0.01 <sup>b</sup>	0.11±0.01 <sup>b</sup>	0.15±0.01 <sup>a</sup>	*
Bursa (g)	0.05±0.01 <sup>c</sup>	0.09±0.06 <sup>b</sup>	0.11±0.01 <sup>b</sup>	0.4±0.01 <sup>a</sup>	*
Gizzard (g)	2.40±0.24	2.45±0.14	2.60±0.25	2.70±0.15	NS
Intestine, (g)	4.70±0.20 <sup>a</sup>	4.40±0.13 <sup>b</sup>	4.20±0.13 <sup>b</sup>	4.10±0.26 <sup>b</sup>	*

Means within row for each item having different superscript differ significantly  
 NS = Not significant \* P ≤ 0.05 \*\* P ≤ 0.01

The same trend was quite evident for WBC's count; it was increased in birds fed Organic Green Culture supplemented diet to 1.2 fold of the control. The corresponding value for birds received diet supplemented with Xylam was 1.03 fold. Heterophils (H), Basophiles(B) and lymphocytes (L)percentage were higher with Organic Green Culture by 9.8, 38.7, 6.6%, respectively, compared to the control. The same trend was noticed with Samu Biogen where heterophils, basophiles and lymphocytes increased by 9.4, 33.3, 3.7%, respectively, compared to the control while Xylam supplemented group showed a little variation compared to the control.

**Table (4): Hematological traits of growing Japanese quails as affected by different experimental treatments.**

Treatments Traits	Control	Xylam	Samu Biogen	Organic Green Culture	Significance
RBC's(X10 <sup>3</sup> /ml)	2.70±0.15 <sup>d</sup>	3.17±0.14 <sup>c</sup>	3.23±0.21 <sup>b</sup>	3.55±0.19 <sup>a</sup>	*
WBC's (X10 <sup>3</sup> /ml)	22.56±0.93 <sup>d</sup>	23.20±0.53 <sup>c</sup>	25.20±0.39 <sup>b</sup>	27.08±0.37 <sup>a</sup>	*
Heterophils(H), (%)	40.16±0.54 <sup>d</sup>	40.83±0.60 <sup>c</sup>	43.93±0.60 <sup>b</sup>	44.08±0.37 <sup>a</sup>	*
Basophiles(B) (%)	3.00±0.55 <sup>b</sup>	3.16±0.36 <sup>b</sup>	4.00±0.30 <sup>a</sup>	4.16±0.60 <sup>a</sup>	*
lymphocytes (L) (%)	40.66±1.05 <sup>d</sup>	41.00±0.77 <sup>c</sup>	42.17±0.76 <sup>b</sup>	43.33±1.64 <sup>a</sup>	**

Means within row for each item having different superscript differ significantly  
 \* P ≤ 0.05 \*\* P ≤ 0.01

**Plasma constituents:**

Table 5 summaries the effect of Organic Green Culture, Samu Biogen, and Xylam supplementation on plasma total protein, albumin, globulin, AST and ALT. It is obvious that there was an interspecific variation in all investigated serum biochemical variables between treated groups and control except AST and ALT.

Plasma total protein increased significantly in birds received Organic Green Culture and Samu Biogen by 41.5% and 22.0%, respectively, compared to the control. At the same time, no significant difference in total protein was detected between Xylam supplemented group and control group. The highest value of plasma albumin was observed in birds received Organic Green Culture followed by Samu Biogen and xylam compared with the control. A similar pattern of response to that described for total protein was noticed for globulin. Glubulin concentration increased with Organic Green Culture by

48.7% followed by 26.1% with Samu Biogen compared to the control. Glubulin in birds received Xylam was statistically differ compared with the control. Plasma AST and ALT were not markedly affected by Xylam, Samu Biogen and Organic Green Culture compared with the control.

**Table (5): Plasma constituents of growing Japanese quails as affected by different experimental treatments.**

Treatments Traits	Control	Xylam	Samu Biogen	Organic Green Culture	Significance
Total protein (g/dl)	2.46±0.06 <sup>c</sup>	2.53±0.12 <sup>b</sup>	3.00±0.02 g <sup>a</sup>	3.40±0.20 g <sup>a</sup>	**
Albumin (g/dl)	1.31±0.06 <sup>c</sup>	1.22±0.06 <sup>b</sup>	1.55±0.04 <sup>b</sup>	1.77±0.20 <sup>a</sup>	*
Glubulin (g/dl)	1.15±0.06 <sup>c</sup>	1.31±0.02 <sup>b</sup>	1.45 ±0.04 <sup>b</sup>	1.71±0.06 <sup>a</sup>	**
AST (u/L)	42.56±2.9	38 ±2.6	37.63 ±3.1	37.40±3.5	NS
ALT (U/L)	1.30±0.22	1.33±0.21	1.16±0.16	1.16±0.16	NS

Means within row for each item having different superscript differ significantly

NS = Not significant

\* P ≤ 0.05

\*\* P ≤ 0.01

Table 6 showed the results of HI titer at 21, 35, and 42 days of age. The results indicated that HI titer was significantly higher in the birds received Organic Green Culture followed by those fed Samu Biogen then those received Xylam as compared with the control group. The HI titer has reached its maximum value at the end of the experiment (42 days) with Organic Green Culture compared with the control.

Bacterial counts as affected by different treatments are demonstrated in Table 7. The lowest bacterial count was observed with Organic Green Culture it decreased by 91.8% followed by Samu Biogen (81.8%) then Xylam supplemented group (76.4%) compared with the control. The same pattern was noticed with *E.Coli* count. The Organic Green Culture supplemented group decreased *E.Coli* count than the other treated group, it decreased by 93.3 % compared with the control while *E.Coli* count decreased by 80.0%, 76.7%, with Samu Biogen and xylem, respectively compared with the control. These results may suggest the antibacterial effect of Organic Green Culture in the intestine.

**Table (6): Hemagglutination inhibition test (HI/ log<sub>2</sub> titers) in growing Japanese quails as affected by different treatments.**

Treatments Traits	Control	Xylam	Samu Biogen	Organic Green Culture	Significance
At 21 day	3.8±0.50 <sup>c</sup>	3.8±0.40 <sup>c</sup>	4.1±0.40 <sup>b</sup>	5.8 ±0.40 <sup>a</sup>	**
At 35 day	3.1±0.30 <sup>c</sup>	4.5±0.30 <sup>b</sup>	4.5±0.30 <sup>b</sup>	5.5±0.30 <sup>a</sup>	**
At 42 day	3.3±0.34 <sup>d</sup>	3.8±0.34 <sup>c</sup>	4.8±0.54 <sup>b</sup>	5.0±0.34 <sup>a</sup>	**

Means within row for each item having different superscript differ significantly

\* P ≤ 0.05

\*\* P ≤ 0.01

**Table (7): Bacterial counts (log<sub>10</sub>/gram of cecal samples) in growing Japanese quails as affected by treatments.**

Treatments Traits	Control	Organic Green Culture	Samu Biogen	Xylam	Significance
Total bacterial count	11 × 10 <sup>4d</sup>	9 × 10 <sup>3a</sup>	20 × 10 <sup>3b</sup>	26 × 10 <sup>3c</sup>	**
Total <i>E. coli</i> count	3 × 10 <sup>4d</sup>	2 × 10 <sup>3a</sup>	6 × 10 <sup>3b</sup>	7 × 10 <sup>3c</sup>	**

Means within row for each item having different superscript differ significantly

\* P ≤ 0.05      \*\* P ≤ 0.01

## DISCUSSION

The experimental treatments used in the current study aimed to improve the nutritional value of corn-soybean based diets. It is well known that poultry do not secrete all kinds of enzymes needed for a complete digestion of the components of feedstuffs. Thus, supplementing poultry diets with enzymes is becoming urgent. The feed enzyme industry focuses primarily on removing the antinutritive effects of non-starch polysaccharides (NSP) from diets (**Annison, 1991**). However, microbial enzymes targeting these polymers have, for this reason, shown highly positive results in both enhancement of performance and reduction of excreta volume and moisture (**Lazaro et al., 2004**).

The current results showed that Organic Green Culture, Samu Biogen and Xylam supplemented to the corn-soybean based diet improved body weight and weight gain compared to the un-supplemented diet. Xylam supplementation improved feed conversion by decreasing feed consumption. Enzymes addition improved feed utilization of feeds as indicated by **El-Husseiny et al. (1995)**. **Friesen et al. (1991)** showed that enzyme supplementation improved feed conversion efficiencies due to the direct effect of enzyme on cereal grain nutritive value. It is demonstrated that enzymes supplementation accelerate the passage of the digesta (**Almirall and Esteve-Garica, 1994**), and therefore, induce a higher feed intake, the decrease in viscosity was parallel to an improvement in feed conversion ratio, energy and starch digestibility (**Choct et al., 1995**). The increase in the energy and nutrient supply might be an essential factor for the intensified growth of birds fed enzyme supplemented diets. **Wu et al., (2004)** pointed out that the positive effects of enzymes supplementation could be attributed to improve nutrients digestibility in young chicks as well as improve the digestion of soluble and insoluble NSP in corn and soybean meal (SBM). Xylanase supplementation tended to increase goblet cell numbers in the duodenum and decrease crypt depth in the jejunum and ileum, this indicate that cell wall degrading enzymes had stimulating effect on absorptive capacity. **Hashish and El-Ghamry (1998)** reported that the effectiveness of enzyme preparation may be due to the improvement in apparent protein digestibility by increasing the activities of proteolytic enzymes in small intestine. **Veldman and Vahi (1994)** showed that viscosity caused by xylans has a detrimental effect on dry matter and mineral absorption in broiler. Xylanases enzymes have been shown to improve the performance of broiler (**Flores et al., 1994 and Choct et al., 1995**), throughout the degrading of viscous non starch polysaccharides starch and pentosan digestibility (**Annison, 1992**), it has been suggested that the improvements caused by the xylanase preparation were due

to a reduction in pentosan concentration in the digestive tract. A partial hydrolysis of the xylan by xylanase supplemented diet results in a large decrease in digesta viscosity which would create an environment more conducive to optimum digestion of crude protein and carbohydrates. Within nutrients needs, ME intake is well documented to influence body composition (Morris, 2004) and performance of growing broiler (Leeson *et al.*, 1996). Palander *et al.*, (2005) reported that the use of enzymes containing  $\beta$ -glucanase-xylanase improved AMEn in wheat, barley and oat based diets for turkeys. The results observed by Adeola and Bedford, (2004) showed that xylanase alone improved TME more in high-viscosity than low-viscosity wheat when fed to the ducks.

Malathi and Devagowda (2001) reported that the level of NSP is up to 29% in SBM and 9% in corn. Both maize and soybean provide targets for NSP enzymes alone or in conjunction with other enzyme classes potentially able to improve nutrient availability. However, Hong *et al.*, (2002) found that enzyme cocktail improved the digestibility of a corn-soybean based diet for ducks. Soybean cells walls contain a very high proportion of pectic polysaccharides (galacturonans and associated arabinogalactans) which account for approximately half of the NSP present. Enzyme targets in soybean are the water-soluble carbohydrates (WSC) which account for 10% by weight of the bean and the pectic polysaccharides that account for a further 10%. Huisman *et al.* (2000) pointed out that disruption of the pectic fraction has a number of potential benefits. These include avoiding any contribution to digesta viscosity of the pectic polysaccharides and ensuring full disruption of the cellular matrix of the meal. Either or both of these effects can significantly improve nitrogen retention. Scholtyssek and Knorr, (1987) have reported some improvement in N retention when diets were supplemented with cellulolytic enzymes preparations. The addition of enzyme complex could have released cell wall encapsulated proteins, which were subsequently utilized. Marsman *et al.* (1997) showed that disruption of the cell-wall matrix by the exogenous glycanases in the upper intestine leads to easy access of the endogenous proteolytic enzyme to digest the entrapped proteins.

Maize, like other cereal grains, has a cellular endosperm with thin endosperm cell walls and a cell wall composition similar to that of wheat. Highly branched arabinoxylans predominate in the endosperm, but mix – linked glucan and a small amount of cellulose are also present. The cell wall forming the outer layers of kernel, (the pericarp and seed coat) are similarly rich in xylans and in cellulose but, unlike other cereals, are not extensively lignified (Saulnier *et al.*, 1995). However, detailed structural analysis of soybean and to lesser extent maize polysaccharides suggests that enzymes known in commercial preparations may be appropriate and effective. Better results could be achieved if formulation included activities effective against the novel structures that have now been identified.

The observed positive effect of enzymes on chick growth could be also attributed to improve gut environment, reduced pathogenic bacteria, i.e. enterobacteria, total anaerobe microbes, and gram-positive cocci and entero cocci and reduced digesta viscosity even if non-viscous grains e.g. maize and sorghum (Cowan, 1995). In this regard, Bedford and Apajalahti (2002) observed that a considerable amount of starch remained encapsulated in the cell walls in the small intestine of chickens; this encapsulation effect seems to be more apparent in non-viscous cereal grains than viscous cereals.

**Brown (1996)** reported that corn starch is not completely digested at the terminal ileum, and their digestion is only in the hindgut. He suggested that some corn starch is somewhat resistant. The enzyme cocktail is expected to act on resistant starch and improve accessibility and solubility of nutrients, thus improving ME and protein digestibility. **Mahagna et al. (1995)** reported a decrease in pancreatic chemotrypsin activity in chicks supplemented with only amylase or protease. Thus exogenous enzymes used in the diets of young chicks would be very beneficial in improving nutrients digestibility either by supplying enzymes that the chicks cannot produce in sufficient quantity by itself or by reducing the requirement for the enzymes, thus making more nutrient and energy available for growth of the chicks at that critical stage (**Olukosi et al., 2007**).

**Zyla et al. (1996)** showed that complete dephosphorylation of corn-SBM diet needs to use an enzyme cocktail rather than one enzyme; the cocktail was more effective than phytase alone at promoting the performance of turkey poults. **Onyango et al. (2005)** reported some improvement in N retention and some amino acids over the low- P diet in broiler chicks receiving corn- soybean based diet supplemented with phytase. The total fecal and ileal digestibility observed by **Olukosi et al., (2007)** showed that both enzyme cocktail and phytase improved P liberation.

Phytate molecule is not the only molecule that is responsible for nutrient's chelating, there is evidence that some soluble fibers, including soluble NSP, are responsible as well (**Frolich, 1990**). It has been shown that soluble NSP and phytic acid are in the aleurone cell layer. **Parkkonen et al. (1997)** reported that xylanase increased the permeability of the aleurone cell wall layer, which is the site of phytate storage. It is possible that enzyme cocktail, by improving aleurone layer permeability, enhances the access of endogenous phytase to phytate molecules, hence improving P digestibility and retention.

With increasing concern about antibiotics resistance, there is an increasing interest in finding alternatives to antibiotics in poultry production. Probiotics are one of the approaches that have a potential to reduce chances of infection in poultry and subsequent contamination of poultry product. The Organic Green Culture and Samu Biogen used in the present work are considered as a commercial feed additive containing probiotics and enzymes in poultry feed. The Organic Green Culture and Samu Biogen induced beneficial effect on broiler performance. The current results are compatible with those reported by **Ahmad (2006)** who pointed out that growth pattern of treated birds showed an increase in weight gain relative to the control. The current data showed also that supplemented chicks consumed less feed than the control. In spite of less feed consumed, treated quails showed a better feed conversion ratio. These results are in harmony with those reported by **Silva et al., (2000)** who mentioned that supplementation with *L. acidophilus* did produce a significant improvement in feed conversion, a feature that could, perhaps, be beneficial with respect to the economic prospects for treatments.

The improvement in live body weight is mainly due to maintaining the beneficial bacteria such as lactobacillus in the intestinal tract of the birds. Lactobacillus in their natural environment competes with the undesirable organisms for space and nutrients as reported by **Jin et al., (1996)**. Lactobacilli are among the indigenous flora colonizing the chick crop. Colonizing bacteria adhere firmly to the mucosal epithelium (**Beachey, 1980**). Useful bacterial growth facilitates the fermentation process in all kind of animals. This

fermentation is of nutritional significant in most animals. The oligo- and polysaccharides have been reported to be responsible for significant changes in the number and metabolism of microflora colonizing the gut because they are not digested in the small intestine and provide substrates for fermentative bacteria (Cummings, 1981). In the chicken crop small amount of starch is broken down by the fermentative process. The bacterial breakdown produces various types of vitamins (Fuller, 1997) and organic acids, which provide energy to the host as well as stimulate the growth (Lan *et al.*, 2003). Ahmad (2004) reported that probiotics have an effect on the main physiological functions of the gastrointestinal tract with an increase of crypt cells proliferation of small intestines leads to more digestion, absorption and propulsion as well as competition for adhesion receptors in the intestine, competition for nutrients, reduction of toxin release and immuostimulatin (Nahashon *et al.*, 1994).

In the current investigation the birds received diet supplemented with Xylam decreased the intestine weight compared with control. These results are compatible with those observed by Brenes *et al.* (1993) who stated that the addition of enzyme reduced small intestine and colon size. The increase in gastrointestinal tract size may, therefore, be an adaptive response to an increase in their need for enzymes. The reduction in the relative organs weight of birds received enzymes is also of direct economic. In the current study, lymphoid organs followed the opposite trend compared to relative organs weight where Organic Green Culture and Samu Biogen induced significant increase in lymphoid organs weights. This increase may be connected to the effect of Organic Green Culture and Samu Biogen on lymphoid organs activity. Measures of immunity that have been commonly used and assessed in poultry are lymphoid organ weights (Pope, 1991) and antibody response to foreign antigens (Klasing, 1998). Lymphoid organs weights are easily measured and reflect the body's ability to provide lymphoid cells during an immune response (Heckert *et al.*, 2002).

In the current study the hematological assessment showed that the treatments may stimulate cellular and humoral immunity as indicated by WBC's counts, HI titer test and globulin concentration. It is well known that cellular immunity is a product of (T) lymphocytes which it forms in the thymus gland (immune cells have the ability of removing the foreign antigenic invader through phagocytosis and macrophages production) as well as spleen; it serves as the site for interaction between foreign antigens and different types of leukocytes (Klasing, 1998). Humoral immunity; is a product of (B) lymphocytes; it is formed in the bursa of fabricius and plays an important role in producing antibodies (circulating antibody, Ab's) that can inactivate the invading agent by agglutination, precipitation, neutralization, and lyses.

The WBC's counts were increased in Organic Green Culture and Samu Biogen treated groups compared to the Xylam treated group and control. This result may indicate that Organic Green Culture and Samu Biogen stimulate the bursa, thymus, and spleen to produce more lymphocytes through the improvement in gut ecology and decreased pathogenic bacteria. This helps the birds to be healthier and leads in turn to increase body weight and lymphoid organs weights and activity as stated by Ali (1999).

It is interesting to know that some viruses have the ability to agglutinate to the RBC's. This character is called Hemagglutination (HI). The principle for HI test depends on the utilization of serum vaccinated birds to

prevent the natural hemagglutination of some viruses as NDV to attach to erythrocytes. It indicated that the higher concentration of antibodies in blood means higher HI titers.

The HI titer test data in the current investigation which is considered as indicator for humoral immunity confirmed that both Organic Green Culture and Samu Biogen increased the antibody production. **Havenaar and Spanhaak (1994)** reported that probiotics stimulate the immunity where flora from probiotics migrate throughout the gut wall and multiply to a limited extent or that antigen may be released by the dead organisms are absorbed and thus stimulate the immune system. **Ahmad (2006)** reported that the improvement in the immune system could be attributed to increase macrophage activity, phagocytoses and the production of antibodies as well as increased the local antibodies at mucosal surfaces.

The study of (**Rubio et al., 1998**) showed that *E. coli* counts in both small and large intestinal sections decreased with respect to control values in broiler fed diets containing lupin meal. On the basis of these results, a part of this investigation was conducted to study the effect of the current treatments on total bacterial and *E.coli* counts in the intestine of growing quails fed corn – soybean based diet supplemented with probiotics.

The results of total bacterial and *E.coli* count observed in the current investigation tended to decrease with Organic Green Culture followed by Samu Biogen and Xylam. These results are in harmony with those observed by **Jin et al., (1996) and Jin et al., (2000)** who reported that propiotics containing Lactobacillus strains have the ability to adhere strongly to the intestinal epithelium of chicken, antagonize and competitively exclude some pathogenic bacteria *in vitro* (**Jin et al., 1998**). It has been known that lactic acid intestinal bacteria inhibit pathogenic bacteria especially *Escherichia coli* which is considered an important pathogen causing bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) as reported by **Salmon et al., (1989)**. **Salem et al. (2004)** indicated that probiotics had a positive effect on lactic acid produce by intestinal bacteria activities and it could improve their inhibition to un-friendly bacteria such as *Escherichia coli*. **Qin et al. (1995)** sustained that lactobacilli protect against potentially harmful bacteria such as salmonella and aid the digestive process. Properly selected cultures of probiotics, such as lactobacillus species, can overcome those Lactobacilli found in the natural flora of the birds and exert inhibitory action towards salmonella in the intestinal tract of chickens. The positive effect of propiotics may be in part due to corn and soybean meal content. Soybean contains very high proportion of pectic polysaccharides (galacturonans and associated arabinogalactans) as the main contributing polysaccharides as well as water – soluble carbohydrates (WSC) which account for 10% by the weight of the bean. Corn also has a cellular endosperm like to that of wheat contains branched arabinoxylans mixed with glucan, small amount of cellulose and xylans which are quite labile and probably highly susceptible to fermentative break-down (**Carre et al., 1989**). This suggests that corn – soybean meal diet, would offer some saccharolytic microflora species colonizing in the gut a suitable substrate to grow. It has been shown *in vitro* that xylo and fructo-oligosaccharides are converted to acids at the highest rate by Lactobacilli and not used by most *clostridia* and *E. coli* among others. **Tannock (1990)** mentioned that broiler chicks are peculiar in that they contain high numbers of lactobacilli particularly in the upper parts of their intestinal tract. This results from the

stratified structure of the non-secretorial mucosa in their crop which provides the appropriate conditions for these species to grow. Its action for, by encouraging the growth of native species of Lactobacilli at the expense of the *clostridia*, a degree of nutritional enhancement could still follow an improvement that is magnified by the concurrent ingestion of Lactobacillus.

## CONCLUSION

It could be concluded that the growth promoting effect of Organic Green Culture, Samu Biogen and Xylam could be attributed to the beneficial components that may exist in the commercial products either enzymatic or probiotics. Both components may act synergistically to increase growth performance and immunity.

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المظاهر التطبيقية لإضافه خليط من الإنزيمات ومعدنات النمو الحيويه إلى علائق السمان

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تهدف هذه الدراسة إلى بيان تأثير إضافة كل من الأنزيمات ومعدنات النمو الحيوية إلى العلائق الأساسية المكونة من الذرة وفول الصويا على أداء إنتاجية السمان اليابانى. وزعت طيور السمان عمر يوم البالغ عددها أربعمائة وثمانون طائر عشوائياً إلى اربعة مجموعات بكل منها ١٢٠ طائراً . غذيت الطيور على العلائق التجريبية المختلفه حيث أحتوت العليقة الأولى على الزيلام (بيتا زيلانيز - الفا اميليز) بمستوى ٥ جرام/ كيلو جرام عليقة وأحتوت العليقة الثانية على الساموبوجين (باسيلس سابنتيلس ماتا - سيلولاز - بروتياز - الفا اميليز - بيتا اميليز) بمعدل ٢ جرام/كيلو جرام عليقة اما العليقة الثالثة فقد إحتوت على الأورجانيك جرين كالشر (ساكارو ميسس سيرفيسيا- لاکتوباسيلس اسيدوفلاس- باسيلس سبتيلس- اسيرجلس اورايزا- بروتياز- اميليز- لياز- سيلولاز- فايينز) بمعدل ١.٥ جرام لكل كيلو جرام عليقة. اظهرت النتائج أن إضافة الأورجانيك جرين كالشر أدت إلى زياده وزن الجسم بمقدار ١٣.٤٪ أما إضافة الساموبوجين والزيلام فقد أدت إلى زياده قدرها ٨.٥ ٪ و ٧.١ ٪ على الترتيب مقارنة بالعليقة الكنترول. أوضحت نتائج اختبارات الذبح أن هناك إختلافات إحصائية ترجع إلى أضافة الأورجانيك جرين كالشر على اوزان القلب وغدة الثيموس والطحال والبرسا مقارنة بالكنترول بينما إضافة الساموبوجين والزيلام أحدثت تأثير متساوى على وزن البرسا والطحال والقلب. أدت إضافة الأورجانيك جرين كالشر إلى زيادة عدد الكرات الدم الحمراء والبيضاء وتبعها المعاملة بالساموبوجين ثم المعاملة بالزيلام مقارنة بالعليقة الكنترول. إضافة الأورجانيك جرين كالشر والساموبوجين أحدثت تأثيراً معنوياً على نسبة البروتين الكلية والألبومين والجلوبيولين مقارنة بالكنترول. لم تؤدي المعاملة بالأورجانيك جرين كالشر والساموبوجين والزيلام إلى حدوث إختلافات معنوية فى تركيز إنزيمات الترانس أمينيز مقارنة بالكنترول. ارتفع النتر المناعى فى الطيور التى تلقت الأورجانيك جرين كالشر وتلاها تلك التى تلقت الساموبوجين ثم الزيلام. سجلت المعاملة بالأورجانيك جرين كالشر اقل عدد بكتيرى كلى وايضا بكتيريا الايشريشيا كولاي وتبعها المعاملة بالساموبوجين ثم المعاملة بالزيلام مقارنة بالكنترول. يقترح من هذه النتائج ان اضافة الانزيمات مع معدنات النمو الحيويه الى العلائق الأساسية المكونه من الذره وفول الصويا تؤدي الي حدوث تحسن في الكفاءة الإنتاجية لطيور السمان.