



Evaluation of *Saccharomyces cerevisiae* Yeast Fermentate and Xylanase in Reduced Energy Diet Fed to Broiler Chicken

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ABSTRACT

Dietary modifications of broiler feed aimed at improving chicken performance, lowering antibiotic use, and reducing feed costs. The study examined the effects of adding *Saccharomyces cerevisiae* yeast fermentate (XPC) and xylanase enzyme to a low-energy diet on broiler chicken growth performance, serum metabolites, intestinal morphology, as well as litter quality. A total of 450 Ross-308, day-old chicks were randomly allotted to 3 diet treatments for 35 days. Group 1 (Gp1) received a basal diet, Gp2 received a low-energy diet (-120kcal/kg), while Gp3 received a low-energy diet (-120kcal/kg) supplemented with both XPC (0.625kg/ton) and xylanase (500g/ton). Following the experiment, chickens were sampled for their intestinal tissues, blood, and cloacal swabs, to determine intestinal morphological indices, serum chemistry, antibody titers, and microbiological counts, respectively. Samples of litter were taken to perform chemical and microbiological analysis. Dietary supplementation with XPC and xylanase to the low-energy broiler diet significantly increased the final body weights, carcass traits, and improved feed conversion ratio (FCR) ($P<0.05$). Additionally, the microbiota of the birds' gut, intestinal morphology and blood biochemical indicators were improved. On an environmental level, the use of dietary XPC and xylanase enhanced nitrogen and phosphorous utilization by the birds and lowered their excretion in broiler manure. Additionally, the microbial load of the litter was reduced ($P<0.05$). We found that feeding XPC and xylanase to broilers fed a low-energy diet (-120kcal/kg) improved broiler performance and enhanced litter quality.

Key words: XPC, Low-energy diet, Litter quality, Gut histomorphology, Blood biochemistry

INTRODUCTION

Recently, there have been eminent concerns regarding the use of feed additives to promote growth, safety, and quality of poultry meat to satisfy increasing consumer demands (Ruiz et al. 2015). In addition to being an antibiotic alternative, yeast and yeast products have numerous benefits for gut physiology, morphology, and microbiology; this is why they are used as a supplement to poultry diets (Zhang et al. 2005). It has been found that providing *Saccharomyces cerevisiae* (SC) to broiler chicks improved their growth potential, ileal mucosal function, and meat quality. Studies have shown the role of SC in boosting digestion and nutrient utilization of birds (Zhang et al. 2005; Hosseini 2011; Awaad et al. 2019). Additionally, SC favored the immune response of broiler

chicks against infections such as *Salmonella* and Newcastle disease (Ghosh et al. 2012; Feye et al. 2019).

The use of exogenous enzymes, such as xylanase, saved an average of 4 billion dollars from the world feed market each year (Adeola and Cowieson 2011). Birds can digest starch, proteins, and lipids, however non-starch polysaccharides (NSP) remain undigested, since they lack hydrolyzing enzymes. When broiler diets were supplemented with enzymes, they performed better and utilized nutrients more efficiently (Bedford and Partridge 2001; Burrows et al. 2002; Walk and Poernama 2019). Exogenous enzymes involved in NSP-hydrolyzing, such as xylanase, are most often applied to diets with low calcium levels, phosphorus, and energy. A corn-based diet, supplemented with xylanase, was reported to improve the digestion of resistant starch and increased access to cellular

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walls. A further benefit, provided by xylanase, is the production of oligosaccharides that are comparable to those found in prebiotics (Bedford and Partridge 2001; Alam et al. 2003; Cowieson and Ravindran 2008; Zargi 2018).

On the other hand, deep litter is a mixture of bedding materials, feathers, and animal droppings. Poultry litter is rich in nutrients such as phosphorous, potassium, nitrogen, and trace minerals required for growing crops. Today's environmental regulations concerning emissions make it essential to employ enhanced management practices to poultry litter (Kelleher et al. 2002 and Cook et al. 2011).

Thus, the present study was designed to evaluate the effect of a low-energy diet supplemented with *Saccharomyces cerevisiae* (XPC) and xylanase on broiler growth performance. Furthermore, carcass traits, gut histomorphology and blood biochemical indices, as well as antibody titers to AI-H5 and NDV vaccines, were analyzed. Additionally, XPC and xylanase were assessed for their effects on cloacal microbial shedding and litter quality.

MATERIALS AND METHODS

Dietary Treatments and Housing

The experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee, Cairo University, Egypt (Vet CU011020202011). The trial was conducted at Cairo University's Faculty of Veterinary Medicine's Animal and Poultry Research Center in Giza, Egypt. 450 Ross-308 chicks were weighed and randomly assigned to 3 dietary treatments. Each diet was fed to 150 broiler chickens for 35 days in three replicates. The three diet treatments were: the Gp1 that received a basal diet; Gp2 that was fed a low-energy diet (-120Kcal/kg); and Gp3 that had a low-energy diet (-120Kcal/kg) fortified with XPC (0.625kg/ton) and xylanase (500g/ton).

Birds were confined in an open floor house with natural ventilation, at 32°C on day one, then gradually decreased in temperature by 2.8°C each week until they reached ambient temperatures. Weekly measurements of feed intake and body weight were conducted. During the experiment, water and feed were provided ad-libitum. The formulations satisfied the recommended nutrient requirements for broiler chickens (NRC 1994). Table 1 presents the physical and chemical compositions of each dietary treatment. Table 2 also shows the vaccination schedule for the birds.

Growth Performance

A weekly body weight (BW), body weight gain (BWG), feed intake and feed conversion rate (FCR) of each bird group was recorded.

Serum Biochemistry

A total of 15 blood samples (5 ml each) were collected from birds in each dietary group on day 35. Birds' blood was received in sealed heparinized, fluoride, and plain vacutainers for plasma and serum. Blood samples were centrifuged at 4000rpm/15 minutes for plasma and serum separation, then stored at -20°C until analysis (Nelson et al. 2020). A range of physiological measurements was carried out on samples including, glucose, lipid profile, liver profile, kidney profile, and antibody titers for NDV and AI.

Plasma and serum samples were analyzed with a commercial kit (Spectrum®, Egyptian Company for Biotechnology). Blood glucose level was determined, according to Tietz (1995). Lipid profile was assessed through the determination of cholesterol, HDL-cholesterol, and Triglycerides according to Ellefson and Caraway (1976) and Stein (1987). LDL-cholesterol was calculated using Friedewald et al. (1972) equation ($LDL-C = TC - [HDL-C + TG/5]$). Very-low-density lipoprotein cholesterol (VLDL-c) was calculated using Friedewald et al. (1972) equation ($VLDL-c = Triglycerides/5$), which was validated by Warnick et al. (1990). Calorimetric analysis of liver profile was carried out by determining aspartate aminotransferase (AST) (Young 1990), total proteins (Gornall et al. 1949) and albumin (Grant et al. 1986). Globulin was calculated by subtracting the total protein from albumin. Albumin/globulin ratio was also measured (A/G). The kidney profile was evaluated by measuring serum uric acid (Fossati et al. 1980) and serum creatinine (Tobias et al. 1962).

Antibody Titers

A hemagglutination inhibition test (HI) was used to determine antibody titers against AI and ND vaccines in serum samples (OIE 2018a, b). A two-fold serial dilution of serum samples was carried out. Four hemagglutination units (HAU) of inactivated AI-H5 and NDV (Lasota) commercial antigens were prepared. A 1% suspension of chicken erythrocytes was used. Results were expressed as mean \log_2 HI titers.

Intestinal Histomorphometry

From each group, 15 birds (5 birds/replicate) were euthanized, and their gastrointestinal tracts were dissected. Middle sections from the duodenum, jejunum, and ileum (about 0.5 cm in length) were excised. Then, general histological structures of duodenal, jejunal, and ileal sections were verified, according to Bancroft and Gamble (2008). Afterwards, the length of the villus was measured from top to bottom. In addition, the intestinal gland depth (crypt) and the thickness of the tunica muscularis were measured. Five stained sections of each segment (duodenum, jejunum, and ileum) were examined per bird in the Faculty of Veterinary Medicine, Cairo University, via Leica Quin 500 analyzer computer system (Leica Microsystems, Switzerland). An automatic calibration was performed to ensure that the measurement units (pixels); produced by the image analyzer, were converted to actual micrometer units. A final magnification of 40x was used to capture images of each sample section. All sections were measured for villus length, crypt depth, and muscularis layer thickness.

Microbiological Examination of Litter Samples and Cloacal Swabs

Total 15 cloacal swabs were collected from each treatment. Also, 500g of mixed litter samples were collected from five sites of each group, sealed in plastic bags, and sent to the laboratory for bacteriological analysis. Then, cloacal swabs were placed into test tubes of 9mL sterilized saline solution, while homogenization of 10g of litter in flasks containing 90mL of sterile saline solution was carried out (Fries et al. 2005). The counts of total

Table 1: Physical and chemical compositions of the three diets for each growing period

Items	Starter (0 to 14 days)			Grower (15 to 28 days)			Finisher (29 to 35 days)		
	Gp1	Gp2	Gp3	Gp1	Gp2	Gp3	Gp1	Gp2	Gp3
Ingredients%									
Yellow corn	55.24	55.85	53.00	59.39	60.15	57.44	63.64	64.15	61.08
Full fat SBM	8.00	2.30	7.50	12.50	6.40	12.00	16.00	11.00	16.50
Sod bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Soybean meal 46%	27.00	34.70	35.79	18.60	27.00	27.00	10.30	18.60	19.00
Corn gluten meal 60%	6.00	3.10	0.00	6.00	3.00	0.00	6.50	3.00	0.00
XPC TM	0.00	0.00	0.0625	0.00	0.00	0.0625	0.00	0.00	0.0625
Xylanase	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.00	0.05
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
NaCl	0.35	0.39	0.35	0.35	0.39	0.35	0.35	0.30	0.35
L-Lysine	0.25	0.15	0.05	0.25	0.15	0.10	0.30	0.15	0.10
DL-Methionine	0.15	0.15	0.20	0.10	0.15	0.20	0.10	0.15	0.20
MCP	0.90	0.90	0.90	0.80	0.80	0.80	0.80	0.75	0.70
Limestone	1.60	1.80	1.59	1.50	1.45	1.49	1.50	1.44	1.45
Quantum blue (Phytase)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Broiler premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chemical analysis:									
ME (Kcal/kg)	3001.05	2880.75	3000.17	3100.63	2980.24	3103.68	3200.24	3080.48	3201.56
Crude Protein (%)	23.17	23.00	23.20	21.11	21.10	21.06	19.14	19.10	19.19
Crude Fat (%)	3.96	2.93	3.76	4.87	3.77	4.67	5.60	4.68	5.56
Calcium (%)	1.00	1.08	1.02	0.94	0.93	0.95	0.93	0.90	0.91
P. Available (%)	0.50	0.48	0.49	0.45	0.44	0.45	0.42	0.41	0.41

Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625kg/ton)+xylanase (500g/ton).

Table 2: Vaccination schedule of the birds

Age (days)	Vaccine	Dose/Route
6 th	Hitchner B1	Eye dropping
18 th	Lasota	Eye dropping
14 th	Infectious Bursal Disease (IBD)	Eye dropping
	Avian Influenza (H5N1)	0.2 ml/bird subcutaneous injection

aerobic and total anaerobic bacteria in *Clostridia* litter and cloacal swabs were determined using nutrient agar and reinforced clostridial medium (RCM) plates, respectively (APHA 1998). After incubation at 37°C for 24 hours, with providing anaerobic conditions for RCM plates, total viable counts (TVC) were obtained. Only plates with fewer than 300 colony forming units (CFU) were counted (Danon-Moshe et al. 1985).

Chemical Examination of Litter

Litter moisture percentage was obtained by drying 10g of litter at 105°C for 24 to 48 hours and calculating the weight difference (Tran et al. 2015). The total nitrogen was determined by using the Kjeldahl method (Jackson 1973). Total phosphorous was determined using ascorbic acid (Houba et al. 1995) and a spectrophotometer at 880 nm (Spectronic 21D).

Statistical Analysis

Data were analyzed using PASW Statistics, Version 18.0. software (SPSS Inc., Chicago, IL, USA). Results were expressed as means and standard errors (SE) and compared by one-way ANOVA. Statistical significance was considered at $P \leq 0.05$.

RESULTS

Growth Performance

The performance of broiler chickens subjected to the three diet treatments is shown in Table 3. Group 3 received a low-energy diet fortified with XPC and xylanase showed

a significant increase in BWG by 14.7% ($P < 0.05$), as well as FCR by -11.6% ($P < 0.05$) when compared to Gp2. Significant differences in feed intake between groups were observed, with XPC and xylanase fortification to the low-energy diet resulted in an increase of 4.3% compared to G1 ($P < 0.05$).

Carcass Traits

The influence of feeding XPC and xylanase on carcass traits is presented in Table 3. Feeding XPC and xylanase with a low-energy diet to birds (Gp3) resulted in significantly greater carcass weights compared with Gp1 and Gp2 ($P < 0.05$). While dressing % was higher for Gp3 and Gp1, however the difference was not significant. Thigh yield was significantly low in Gp3 compared with other groups ($P < 0.05$). Despite this, the drumstick weight didn't show significant differences among the three diet treatments.

Serum Biochemistry

The results of broilers' blood biochemistry are presented in Table 4. XPC and xylanase in the low-energy diet (Gp3) resulted in significantly higher glucose concentrations than in Gp1 ($P < 0.05$). However, lipid profile (cholesterol, triglycerides, HDL, LDL and VLDL concentrations), liver function (total protein, albumin, globulin) and kidney function (creatinine and uric acid concentrations) showed no significant variations between Gp3 and Gp1 ($P > 0.05$). Further, glucose, lipid profile, total protein, albumin, and globulin levels of Gp3 were significantly higher than those of Gp2 ($P < 0.05$).

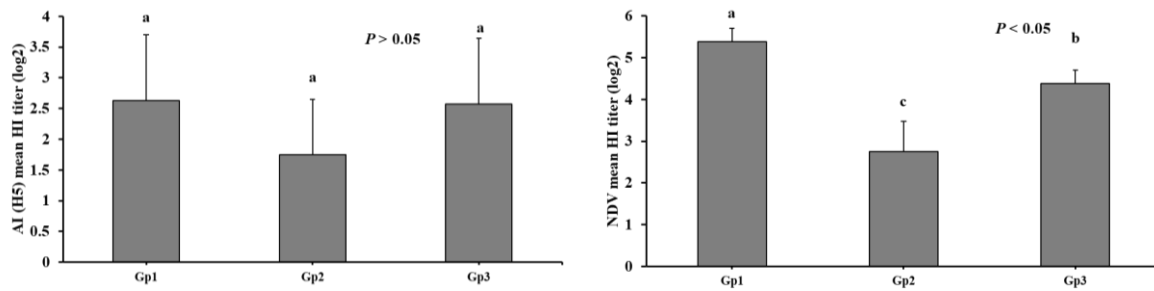


Fig. 1: Mean HI antibody titers against AI-H5 and ND after vaccination of broilers fed diets with different energy levels supplemented with XPC and xylanase; Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625 kg/ton)+xylanase (500g/ton); ^{a,b,c}Different superscripts indicate significant difference at $P < 0.05$.

Table 3: Effects of yeast fermentate (XPC) and NSP-hydrolyzing enzyme (xylanase) on the growth performance and carcass traits of broiler chickens

Items	Gp1	Gp2	Gp3
Growth performance			
Initial weight (g)	44	44	44
Final weight (g)	1990.4±42.66b	1782.4±49.62a	2037.6±22.58b
Body weight gain (g)	1946.0±42.66b	1738.0±49.62a	1994.0±22.58b
Feed intake (g)	3370.0±0.71a	3457.0±0.69b	3515.0±0.75c
FCR* (g/g)	1.74±0.02a	1.99±0.03b	1.76±0.01a
Carcass traits			
Carcass weight	1890.33±24.87a	1854.67±27.96a	2041.33±17.21b
Dressing %	81.11±1.29a	79.07±1.10a	81.95±0.75a
Breast (%)	28.60±0.42a	28.18±0.66a	28.58±0.47a
Thigh (%)	26.49±0.59a	26.49±1.06a	22.70±0.57b
Drumstick (%)	15.99±0.44a	16.32±0.61a	17.13±0.21a

Mean±SE with different alphabets within the same row differ significantly ($P < 0.05$); FCR: Feed conversion ratio. Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625kg/ton)+xylanase (500g/ton).

Table 4: Effects of yeast fermentate and NSP-hydrolyzing enzyme on plasma biochemistry of broiler chickens

Parameters	Gp	Gp2	Gp3
Glucose (mg/dL)	233.51±2.55a	219.89±4.04b	242.92±2.91c
Cholesterol (mg/dL)	149.90±4.72a	133.39±3.97b	149.82±3.78a
Triglycerides (mg/dL)	55.64±1.22a	42.65±1.9b	53.88±1.57a
HDL (mg/dL)	79.61±1.81a	71.14±3.18b	80.03±1.67a
LDL (mg/dL)	59.17±4.67a	53.73±4.91a	59.01±3.02a
VLDL (mg/dL)	11.13±0.24a	8.53±0.39b	10.78±0.31a
GOT (IU/L)	71.30±2.30a	49.41±2.33b	55.98±2.94b
Total Protein (g/dL)	3.90±0.15a	3.42±0.09b	3.91±0.07a
Albumin (g/dL)	1.77±0.02a	1.66±0.02b	1.73±0.02a
Globulin (g/dL)	2.13±0.15a	1.76±0.09b	2.18±0.07a
A/G	0.90±0.07ab	0.99±0.07a	0.81±0.03b
Creatinine (mg/dL)	1.30±0.05a	1.21±0.03a	1.19±0.04a
Uric acid (mg/dL)	4.87±0.32a	3.22±0.28b	4.52±0.51a

Mean±SE with different alphabets within the same row differ significantly ($P < 0.05$). Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625kg/MT)+xylanase (500g/MT).

Antibody Titer

The effects of feeding XPC and xylanase on broilers' antibody response to AI and ND vaccines are presented in Fig. 1. Results noted that broilers' antibody titers to AI-H5 and NDV vaccines remained the highest in Gp1 (fed on the basal diet) and Gp3 ($P < 0.05$).

Intestinal Histomorphometry

Table 5 presents the histomorphometric analysis of the small intestine in the three groups. There were significant differences between Gp2, Gp3, and Gp1 in villi length, crypt depth, and muscular thickness ($P < 0.05$). Also, a significant difference was observed in villi length and crypt depth of Jejunum between Gp3, Gp2, and Gp1 ($P < 0.05$). There was a significant difference in villi length between Gp3 and Gp2 in the ileum ($P < 0.05$).

As illustrated in Fig. 2, birds in Gp1 showed a normal microscopic structure of the small intestine. On the other hand, some duodenal intestinal villi of Gp2 showed epithelial desquamations, while others looked atrophied (Fig. 2b). Compared to Gp2, Gp3 showed improvement in the intestinal villi length (v) and crypt depth. In some sections, there were lymphocytic infiltrations in the lamina propria (arrow) Fig. 2c.

Litter Quality and Cloacal Shedding

Figure 3 and Table 6 demonstrate the effect of feeding XPC and xylanase on deep litter quality. Litter of Gp1 showed higher aerobic bacterial counts than litters of other groups ($P < 0.05$), as well as the anaerobic bacterial counts ($P = 0.05$). Cloacal shedding of Gp1 showed the highest aerobic ($P < 0.05$) and anaerobic bacterial counts ($P < 0.05$).



Fig. 2: A photomicrograph of the duodenum: a) The control group (Gp1) showing normal histological picture for the intestinal villi (V), and tunica muscularis (M), b) Gp2 showing some villi with desquamated epithelium, and c) Gp3 showing villus height (VH), tunica muscularis (M) return to its normal histological structure. H&E stain; 40X.

Table 5: Histomorphometry of small intestine of broilers fed yeast fermentate and NSP-hydrolyzing enzyme

Parameters	Gp1	Gp2	Gp3
Duodenum			
Villi length	2192.69±78.36a	1599.34±90.76b	1803.79±110.83c
Crypt depth	108.48±26.09a	137.34±17.67b	122.47±15.00c
Tunica musculosa	277.61±47.71a	166.60±15.85b	323.05±89.04c
Jejunum			
Villi length	1037.11±41.40a	846.06±50.41b	933.89±39.82c
Crypt depth	92.54±29.07a	167.61±16.77b	132.96±20.92c
Tunica musculosa	319.37±34.84a	283.65±37.60b	319.95±37.34a
Ileum			
Villi length	833.07±34.13a	581.88±60.85b	677.01±25.95c
Crypt depth	249.09±26.71a	203.76±53.19b	204.46±48.72b
Tunica musculosa	394.92±41.45a	351.07±63.57b	344.58±61.49b

Mean±SE with different alphabets within the same row differ significantly ($P<0.05$). Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625kg/ton)+xylanase (500g/ton).

Table 6: Effects of yeast fermentate (XPC) and NSP-hydrolyzing enzyme (xylanase) on the microbial load of litter samples and cloacal swabs of broiler chickens

Items	Gp1	Gp2	Gp3
Deep litter			
Aerobic count ($\times 10^{11}$)	280.33±18.22a	7.13±0.61 b	7.87±0.64 b
Clostridial count ($\times 10^{14}$)	66.00±12.49a	26.33±1.45b	18.67±1.20b
Cloacal swabs			
Aerobic count ($\times 10^9$)	5.40±0.72a	1.80±0.23b	0.41±0.04b
Anaerobic count ($\times 10^{10}$)	49.67±2.89a	35.12±1.92b	4.15±0.39c

Mean cfu/mL±SE with different alphabets within the same row differ significantly ($P<0.05$). Gp1: Basal diet; Gp2: Diet with reduced 120kcal/kg; Gp3: fed on diet with reduced 120kcal/kg+XPC (YF 0.625kg/ton)+xylanase (500g/ton).

Gp3 had the lowest bacterial counts for cloacal shedding. Results of this study showed a significant effect of a low-energy diet supplemented with XPC and xylanase enzyme on lowering the excreted nitrogen and phosphorus contents of litter ($P<0.05$) (Fig.4). Gp2 recorded the highest moisture content, while Gp3 recorded the lowest moisture level ($P<0.05$).

DISCUSSION

Poultry producers remain focused on developing an optimal dietary formula that is cost-effective and enhances birds' performance. In the poultry industry, supplements such as *Saccharomyces cerevisiae* fermentation products (XPC) and non-starch polysaccharide (NSP) hydrolyzing enzymes (xylanase) are widely used. Despite the low-energy diet, XPC with xylanase improved broiler growth performance parameters. Modifying diet can enhance nutrient digestibility and influence manure characteristics (Pierzynski and Gehl 2005). The yeast product XPC acts as an alternative to antibiotics (Morales-Lopez et al. 2009). The exogenous enzyme xylanase enhances enzyme activity in the pancreas and intestinal tract (Horvatovic et al. 2015).

Previous studies indicated that yeast culture and xylanase supplements in broiler diets achieved significant improvements in body weight gain (BWG) and FCR at different ages (Gao et al. 2008; Esmailipour et al. 2011; Fathi et al. 2012; Zhang et al. 2014; Raza et al. 2019). Furthermore, XPC has been shown to enhance feed intake and lower feed conversion ratio (Roto et al. 2017). In contrast, Morales-Lopez et al. (2009) did not find an effect of yeast products on broiler growth performance and recommended evaluating each yeast formulation product individually. According to Walk and Poernam (2019), supplementation with xylanase of reduced energy diets led to higher feed intake and BWG than birds fed with a reduced energy diet; and comparable to birds fed on a basal diet ($P<0.05$). In addition, Cowieson and Ravindran (2008) have noted that xylanase supplemented corn-soy diets improved BWG and FCR of broilers whether they maintained adequate nutrition or had reduced energy and essential amino acids.

The carcass, dressing, and breast weights were significantly improved and reported the highest values in the birds fed a low-energy diet fortified with XPC and xylanase, while the thigh and drumstick weights did not

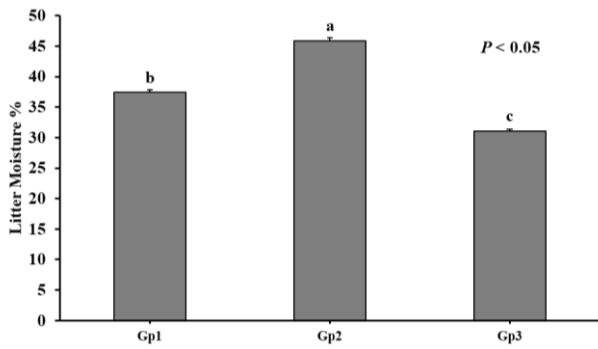


Fig. 3: Litter moisture% of broiler groups fed diets with different energy levels supplemented with XPC and xylanase; Gp1: Basal diet; Gp2: Diet with reduced 120 kcal/kg; Gp3: fed on diet with reduced 120 kcal/kg + XPC (YF 0.625 Kg/MT) + Xylanase (500g/MT); ^{a,b,c} Different superscripts indicate significant difference at $P < 0.05$.

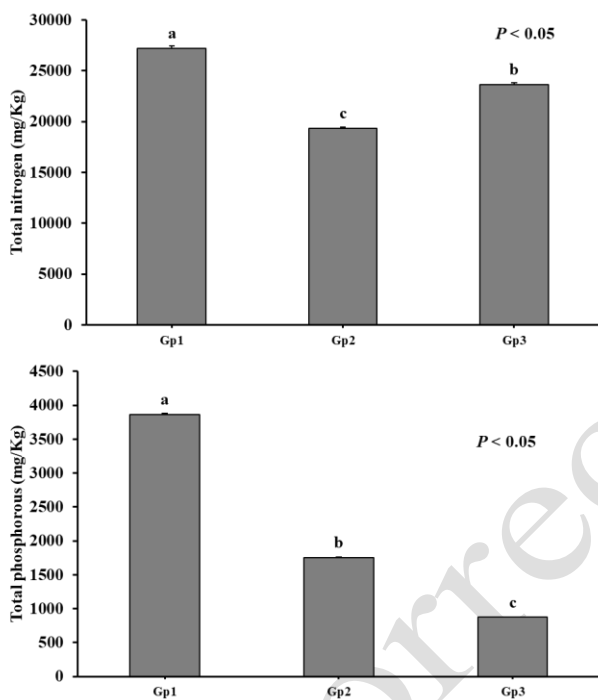


Fig. 4: Litter total nitrogen and phosphorous levels of broiler groups fed diets with different energy levels supplemented with XPC and xylanase; Gp1: Basal diet; Gp2: Diet with reduced 120 kcal/kg; Gp3: fed on diet with reduced 120 kcal/kg + XPC (YF 0.625 Kg/MT) + Xylanase (500g/MT); ^{a,b,c} Different superscripts indicate significant difference at $P < 0.05$.

improve. The characteristics of the carcass influence consumers' decisions about buying chicken meat. A bird's carcass weight is associated with its feeding and nutrition level (Cardoso et al. 2011), as adequate nutrition is associated with enhanced muscle deposition. According to Oyeagu et al. (2019), birds fed xylanase generally had improved carcass traits when compared with birds fed other diet treatments. According to Alam et al. (2003), Wang et al. (2005) and Hajati (2010), adding exogenous enzymes to broiler diets enhanced carcass yield by increasing tissue (muscle) development in the carcass and breast muscle.

The blood biochemical indices of birds who received the low-energy diet fortified with XPC and xylanase were similar to birds fed the basal diet. XPC and xylanase were

shown to be effective in substituting the low energy content of poultry diets. Xylanase works by decreasing the viscosity of digesta, increasing the digestibility of crude protein, starch, soluble and insoluble NSP (Zhang et al. 2014). Viscous digesta provides an environment that is favorable to anaerobes and gram-negative bacteria. Yaghobfar and Kalantar (2017) found that these bacteria boost the production of volatile fatty acids, which lowers the pH and the absorption of digesta. Decreased viscosity leads to improved protein digestibility, feed conversion, energy metabolism, and body weight gain. Xylanase also increases amino acid digestibility in the ileum by about 15% (Raza et al. 2019). Furthermore, xylanase stimulates the secretion of cholecystokinin (CCK). Also, it increases pancreatic trypsin, amylase, and lipase genes expression and increases pancreatic digestive enzyme activity (Yuan et al. 2017). XPC may also contribute to superior physiological indices by reducing physiological stress, whether it was caused by heat stress or normal rearing conditions, by eliminating the free radical in the cells (Nelson et al. 2020). Additionally, XPC has been reported to improve immunity against pathogens (like *Salmonella*) as well as aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* (Osweiler et al. 2010; Feye et al. 2019).

Another significant finding of adding XPC and xylanase to low-energy diets was the improved broilers' immune responses following vaccination against AI-H5 and NDV (Fig. 1). Recent research revealed that raising the dietary XPC provoked the anti-viral antibodies to NDV (Gao et al. 2008; Jensen et al. 2008). But Silva et al. (2009) and Hedayati et al. (2014) declared that yeast extract supplementation did not improve the humoral immune response against NDV and Avian Influenza (AI) in broilers. Due to routine vaccinations, vaccinated hens passed maternally derived antibodies (MDAs) against AI and ND viruses to their chicks through egg yolks (Brambell 1970; Hamal et al. 2006). After 2 to 3 weeks of age, MDA naturally decreases in chicks (Hamal et al. 2006). MDA can protect chicks from clinical illnesses (Mast and Goddeeris, 1999), but may also interfere with vaccine efficacy and decrease its efficiency for NDV (Rauw et al. 2009; van Eck et al. 1991; Kapczynski and King 2005) and AI vaccines (Maas et al. 2011; Kim et al. 2010; Forrest et al. 2013; Lardinois et al. 2016).

Histomorphometry analysis of the small intestine revealed that fortifying the low-energy diet with XPC and xylanase significantly developed the villi length of the duodenum, jejunum, and ileum, compared to the bird group of non-supplemented low-energy diet. Also, XPC and xylanase showed improvement in the histological feature of the duodenum. In the same concern, Gao et al. (2008) and Akhavan-Salamat et al. (2011) mentioned that XPC improved gut morphological features, which in turn could improve nutrient absorption. Xylanase not only improved physiological functions but also lead to morphological changes in the gastrointestinal tract. Supplementation of xylanase increases the digestive and absorptive abilities of the intestinal tract because of the increased villus height, absorption surface, expression of brush border enzymes, and nutrient transport systems (Amat et al. 1996).

The results showed that xylanase and XPC supplementation to a low-energy diet affected the total nitrogen (N) content of litter compared to birds fed the

basal diet (Fig. 4). In poultry litter, urea and uric acid are broken down by microbes, resulting in an 80% loss of nitrogen (N) as ammonia (NH₃) (Kelleher et al. 2002; Ritz et al. 2004). The successful modification of poultry diets could help decrease ammonia emissions in the environment and reduce ammonia's harmful effects on poultry. Dietary phosphorus (P) is a costly component of broiler diets, and excessive application of litter phosphorus to soil leads to runoff and phosphorus pollution of surface and groundwater (Dankowiakowska et al. 2013). Xylanase and XPC fortification to a low-energy diet increased phosphorus utilization and reduced its excretion in broiler manure, which reduced environmental pollution and feed costs. It has been found that nutritional strategies can be used to increase the ability of animals to utilize nutrients, which in turn can lower the excretion of elements into the environment (Lin et al. 2017; Awaad et al. 2019). To achieve environmental safety and economic feasibility, Lin et al. (2017) emphasized the importance of integrating nutritional approaches into production systems.

On the other hand, birds fed on the XPC and xylanase with the low-energy diet produced the least wet litter (Fig. 3). Wetting of poultry litter occurs when the frequency of excreted water (urine and feces) exceeds the rate of evaporation (Collett 2007). Poultry does not have the enzyme repertoire necessary to digest soluble non-starch polysaccharides (NSP); as a result, NSP increases fecal moisture and causes wet litter (Bedford 2006). NSP also forms high molecular weight complex polymers that increase the viscosity of the intestinal aqueous phase and upsetting the digestive tract (Choct and Annison 1992; Bedford 1996). This finding confirmed the effect of the NSP- hydrolyzing enzyme (xylanase) on reducing litter moisture; when compared with other dietary treatments.

Birds given a low-energy diet fortified with XPC and xylanase exhibited lower litter bacterial loads than those given non-supplemented diets. The microbial quality of poultry litter can significantly affect the intestinal microbiota of the birds. Earlier studies predicted that bacteria would cycle between poultry litter and the intestines of birds (Cressman et al. 2010). Viscous intestinal digesta provides an environment that is favorable to anaerobes (Yaghobfar and Kalantar 2017). Previous studies revealed that such feed supplements could suppress foodborne pathogens by modifying the gastrointestinal microbial population (Ibukic et al. 2012; Ricke et al. 2020). Xylanase could increase beneficial bacteria like *Lactobacillus* and reduce pathogenic bacteria like *Clostridium perfringens* by degrading NSP (Raza et al. 2019).

Conclusion

Fortifications of broiler chicken diet with xylanase at 500g/ton and XPC at 625g/ton could lead to improved overall birds' performance, as well as carcass traits. Furthermore, both xylanase and XPC improved broilers' gut health, as measured by increased villi length and reduced gastrointestinal microbial populations. The biochemical indices of blood confirmed that xylanase and XPC replaced the lowered energy in the broiler diet. From an environmental sustainability perspective, XPC and xylanase could enhance the chemical properties of deep litter.

Author's Contribution

All authors participated in the aim of the work and designing of the study; Khaled N. E. Fahmy: Performed the experimental design, Methodology, Supervision, Data curation, review, and editing; Elshaimaa Ismael: Microbiological and Chemical analysis, Serological analysis, Data curation; Eman M. Ismail and Hanan S. Khalefa: Microbiological and Chemical testing; Ebtihal M. M. Elleithy: histomorphometric analysis; Sherif H. Elmosalamy: Blood biochemistry; Sherif Marouf: Bacteriological analysis. All authors shared in writing and revising the manuscript.

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