



## RESEARCH ARTICLE

### Effect of Yeast Derived Carbohydrate Fraction on Growth Performance, Apparent Metabolizable Energy, Mineral Retention and Gut Histomorphology of Broilers during Starter Phase

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#### ABSTRACT

The effect of a specific strain of yeast derived carbohydrate fractions (YCF; Actigen<sup>®</sup>) at two different levels was investigated on broilers performance, apparent metabolizable energy, mineral retention and gut histomorphology. One hundred and eighty, day-old male broiler (Cobb) were randomly allotted to replicated (n=4; 15 birds/replicate) dietary treatments (n=3) i.e. YCF-0, YCF-0.8 and YCF-1. The YCF-0 served as control and to the others YCF was added at the rate 0.8 and 1g kg<sup>-1</sup> of diet, respectively. Birds were reared in cages in an open sided house and had free access to feed and water. Significantly higher feed intake (845.26±3.30g), body weight gains (653.13±4.50g) and improved FCR (1.29±0.03) were observed in group YCF-1 compared to the other dietary groups. Difference in small intestinal length was insignificant. However, goblet cells count and villus height was increased (P<0.05) in birds supplemented with the higher level of YCF. There was an increase (P<0.05) in AME (8.22%) and retention of phosphorus (10.8%) and calcium (9.7%) by birds in group YCF-1. Relative weight of bursa, thymus and spleen improved in supplemented birds. It was concluded that supplementation of carbohydrate fraction could effectively enhance production performance, gut histomorphology and nutrient utilization by birds during starter phase.

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#### INTRODUCTION

Use of antibiotics at sub-therapeutic level is still a common practice in the developing world as a strategy to improve feed utilization and body weight gain. This however, has been banned recently by USA and other EU countries due to serious health concerns and the development of microbial resistance (Castanon, 2007). Poultry scientists are striving hard on improving poultry genetics, management, health and nutrition to maximize bird's performance. New developments have been made to replace antibiotics as growth promoter in poultry production and to surmount emerging microbial resistance to different antibiotics (Ivkovic *et al.*, 2012). The use of poultry feed enzymes, probiotics, prebiotics, synbiotic and phytobiotics in poultry production have all shown promising results with better production performance end product quality (Pelicano *et al.*, 2004). Yeast (*Saccharomyces cerevisiae*) can not only serve as feed

ingredient but certain cell components (mannans) have shown to possess growth promoting, immunomodulatory, toxin binding impacts and reducing pathogenic microorganisms in the intestinal tract (Gallo and Masoero, 2010; Ivkovic *et al.*, 2012). Reduced pathogenic microbial load in the gastrointestinal tract have been reported to improve gut functions and nutrient digestibility. Numerous studies (for example Miguel *et al.*, 2004) have reported that mannanoligosaccharides isolated from yeast can improve poultry health and production. However, previously crude mannanoligosaccharides (MOS) was only used with some other cell wall fractions (Ivkovic *et al.*, 2012) that did not reflect fully the positive role of MOS. With the applications of modern genetic technology a more refined, concentrated and pure form of MOS has been isolated from a specific strain of yeast (Actigen<sup>®</sup>, Alltech Inc) possessing with more beneficial implications (Ivkovic *et al.*, 2012) compared to crude MOS (Brümmer *et al.*, 2010). Few studies with equivocal outcomes have

been conducted to assess the efficacy of pure yeast carbohydrate fractions (Actigen<sup>®</sup>, Alltech Inc). Moreover, beneficial impacts were assessed mostly during finisher phase (0-42) of broiler production. Poultry growers are however facing tremendous problems during the starter period of broiler production. Birds during early stage of life are more prone to various stresses that can badly affect growth performance, immunity and digestion due to less development of the different body physiological functions. Also no research work has monitored the role of Actigen<sup>®</sup> on improving apparent metabolizable energy and mineral retention during starter phase (0-21 days) of life. This research work was therefore planned to examine the beneficial impacts of carbohydrate fraction isolated from a specific strain of yeast (Actigen<sup>®</sup>, Alltech Inc) in maize-soybean based diet from 1-21 days on growth performance, apparent metabolizable energy of diet, lower ileum histomorphology and calcium and phosphorus retention of broiler birds.

## MATERIALS AND METHODS

**Ethical considerations:** This research study was pre-approved by the departmental Board of Studies meeting for procedures involving live birds handling and welfare.

**Bird's husbandry and experimental diets:** A total of 180, day-old male broilers (Cobb) were randomly allocated to three replicated (n=4) dietary treatments i.e. YCF-0, YCF-0.8 and YCF-1 with 15 birds per replicate. Birds were reared in open sided house at optimal environmental (Temp: 95-75°F; RH: 65-60%) and management conditions. All birds had an *ad-libitum* access to fresh water and corn-soybean meal based mash diet (22% CP and 2960 MJ Kg<sup>-1</sup>). Actigen<sup>®</sup> (Alltech, Inc. USA) was added in diets at rate of 0.8 (YCF-0.8) and 1 (YCF-1) g/ kg of diet, YCF-0 served as control. Experimental diets were stored in the cool room (4°C) before the start of research.

**Performance data:** Feed intake was determined by offering known quantity of feed and measuring refused feed daily. Bird's weight were measured on day first and then at the end of each week for 21 days. Feed conversion ratio (FCR) was determined and adjustment was made for mortality [(FCR = weight of feed consumed per weight gain of survivors), and was adjusted for mortality (AFCR = weight of feed consumed)/ (weight gain of survivors + weight gain of mortalities)]. Weight of the spleen, bursa of Fabricius, and thymus was measured after removing and stripping of adhering tissues. Relative organ weight was calculated as percentages of body weight = [(organ weight / body weight) × 100]

Two birds per replicate were weighed, killed, skinned off and edible and non-edible parts were removed. Carcass was weighed and expressed in term of percentage. Dressing Percentage = (Dressed weight / Live weight) × 100

**Lower ileum histomorphology:** On day-21, one cm section of the middle of the lower ileum was detached, washed in physiological saline solution, and fixed in 10% formalin (buffered) from two birds in each replicate.

These tissues were washed and dehydrated in different dilutions of alcohol and fixed in paraffin. Luminal contents of the intestinal sections were flushed with cold PBS and were then fixed in 10% formalin (buffered) for 3 days. The fixed intestinal segments were sliced into 5-mm segments and fixed in paraffin. These samples were mounted onto slides after cutting up to 5 µm in thickness. The slides were stained using H & E stain and were observed under microscope. The villus area was obtained from 4 villi/3 tissues per slide and the goblet cells count was taken per villus. The mean number of goblet cells per bird was obtained from 20 readings of goblet cell count.

**Determination of apparent metabolizable energy (AME):** On day-16, 5 birds from each replicate were shifted to 16 metabolic cages (3L x 3W x 2H ft). Birds were offered known amount of feed daily and fresh feces were collected every next morning, weighed and freezed for four days. Gross energy (GE) of the diets and excreta was determined using an adiabatic bomb calorimeter (IKA<sup>®</sup> WERKE, USA) standardized with benzoic acid. Approximately one gram of sample was pressed to a tablet in Pellet Press (Parr Instrument Co, USA), re-weighed and transferred to VM crucible for combustion in the adiabatic bomb calorimeter. Gross energy for the samples was recorded.

### Calculations:

$$AME = \frac{(\text{Energy intake} - \text{Energy lost})}{\text{Feed intake}}$$

**Determination of Ca and P:** Dry samples of feed and feces were acid digested for mineral analyses as described by Sultan *et al.* (2014) using Atomic Absorption Spectrometer.

**Data analysis:** Data was analyzed using standard procedure of Analysis of Variance (ANOVA) in complete randomized design (CRD) and means were compared using Fisher LSD test (SAS, 2004).

## RESULTS

Mean feed intake (845.26±3.3g), body weight (653.13±4.5) and FCR (1.29±0.03) were significantly improved (P<0.05) by birds in group YCF-1 that received the highest level of Actigen<sup>®</sup> compared to YCF-0.8 and YCF-0 (Table 1). Difference between the later two groups was however insignificant. No significant difference was observed in the mean intestinal length (Table 2) of birds among different dietary groups. Goblet cell count and villus heights in the small intestine of birds were significantly increased (Table 2, Fig 1) with Actigen<sup>®</sup> supplementation in YCF-1 and YCF-0.8, respectively. No significant alteration in the mean intestinal length was noted with Actigen<sup>®</sup> supplementation. Apparent metabolizable energy and digestibility coefficients of calcium and phosphorus of broiler birds in different experimental and control groups (Table 3) were significantly altered. Birds in group YCF-1 had enhanced (7.5%) apparent metabolizable energy (13.69±0.08 MJ/kg) to other groups. There was only a numerical increase (2.32%) in the AME of birds in group YCF-0.8

**Table 1:** Effect of yeast carbohydrate fraction (Actigen®) on mean feed intake, body weight gain and FCR of broiler birds during starter phase

Groups	Feed Intake (g)	Weight gain (g)	FCR
YCF-0	795.28±4.1 <sup>b</sup>	575.72±4.2 <sup>b</sup>	1.38±0.03 <sup>b</sup>
YCF- 0.8	794.07±3.9 <sup>b</sup>	590.99±5.1 <sup>b</sup>	1.34±0.02 <sup>b</sup>
YCF-1	845.26±3.3 <sup>a</sup>	653.13±4.5 <sup>a</sup>	1.29±0.03 <sup>a</sup>

Values (mean±SE) carrying different superscripts within a column differ significantly (P<0.05). YCF-0: control); YCF-0.8: 0.8g Actigen® kg<sup>-1</sup> diet; YCF-1: 1g Actigen® kg<sup>-1</sup> diet.

**Table 2:** Effect of yeast carbohydrate fraction (Actigen®) on mean intestinal length, goblet cells count and villus height of broiler birds at day-21

Groups	Intestinal length (cm)	Goblet cells (n)	Villus height(µm)
YCF-0	53.00±0.823	94.30±1.086 <sup>c</sup>	76±0.000 <sup>b</sup>
YCF- 0.8	53.50±0.823	111.95±5.568 <sup>b</sup>	96±0.000 <sup>b</sup>
YCF-1	54.13±0.398	141.35±0.912 <sup>a</sup>	133±0.022 <sup>a</sup>

Values (mean±SE) carrying different superscripts within a column differ significantly (P<0.05). YCF-0: control); YCF-0.8: 0.8g Actigen® kg<sup>-1</sup> diet; YCF-1: 1g Actigen® kg<sup>-1</sup> diet.

**Table 3:** Effect of yeast carbohydrate fraction (Actigen®) on the digestibility of Ca, P and apparent metabolizable energy by broiler birds at day-21

Groups	AME (MJ/kg)	Digestibility coefficient	
		Ca	P
YCF-0	12.65±0.07 <sup>b</sup>	0.74±0.01 <sup>b</sup>	0.72±0.01 <sup>b</sup>
YCF- 0.8	12.95±0.08 <sup>b</sup>	0.74±0.02 <sup>b</sup>	0.72±0.02 <sup>b</sup>
YCF-1	13.69±0.08 <sup>a</sup>	0.82±0.01 <sup>a</sup>	0.79±0.01 <sup>a</sup>

Values (mean±SE) carrying different superscripts within a column differ significantly (P<0.05). YCF-0: control); YCF-0.8: 0.8g Actigen® kg<sup>-1</sup> diet; YCF-1: 1g Actigen® kg<sup>-1</sup> diet.

**Table 4:** Effect of yeast carbohydrate fraction (Actigen®) on the relative weight of lymphoid organs relative to body weight and carcass yield (%) of broiler birds at day 21

Groups	Bursa of fabrics	Thymus	Spleen	Carcass yield (%)
YCF-0	0.178±0.002 <sup>b</sup>	0.26±0.001 <sup>b</sup>	0.071±0.002 <sup>b</sup>	56.19±0.607 <sup>b</sup>
YCF- 0.8	0.197±0.001 <sup>b</sup>	0.28±0.001 <sup>a</sup>	0.079±0.001 <sup>b</sup>	57.74±0.351 <sup>b</sup>
YCF-1	0.264±0.009 <sup>a</sup>	0.28±0.001 <sup>a</sup>	0.089±0.001 <sup>a</sup>	60.85±0.152 <sup>a</sup>

Values (mean±SE) carrying different superscripts within a column differ significantly (P<0.05). YCF-0: control); YCF-0.8: 0.8g Actigen® kg<sup>-1</sup> diet; YCF-1: 1g Actigen® kg<sup>-1</sup> diet.

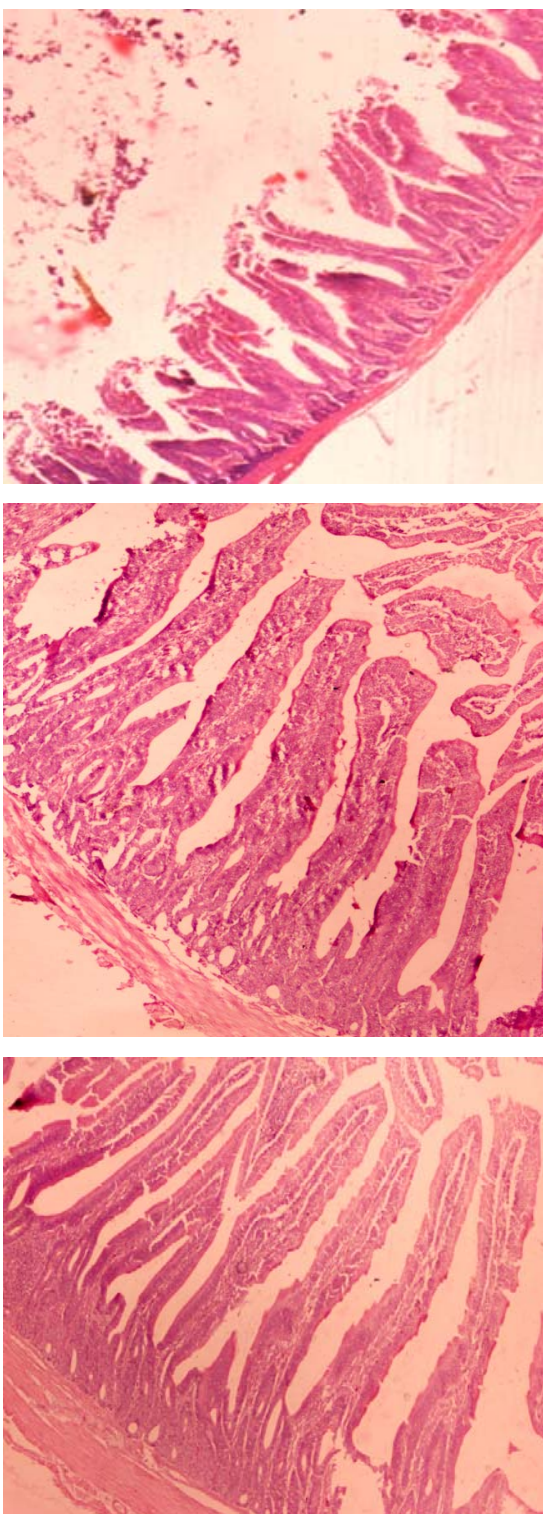
(12.95±0.08MJ/kg) compared to YCF-0. Similarly, difference in the digestibility coefficients of Ca and P was insignificant between group YCF-0.8 and YCF-0. However, the impact of Actigen at higher inclusion level in diet (group YCF-1) was significant to increase Ca (0.79) and P (0.82) uptake by the bird. Birds in this group retained 8.9% and 9.8% more Ca and P, respectively, compared to other two groups. Relative weights of lymphoid organs to body weight were significantly higher in birds (YCF-1) that received higher dose (1g/kg of diet) of Actigen®. Improvement in the weight of bursa of Fabricius was (0.264 %), thymus (0.27%) and spleen (0.089%) by birds in group YCF-1 to other YCF-0. Difference between YCF-1 and YCF-0.8 was however insignificant for thymus. Similarly no significant difference was found between YCF-0.8 and control group for bursa of Fabricius and spleen (Table 4).

## DISCUSSION

In the current study, performance traits improved significantly in YCF-1. Yeast carbohydrate fraction that could potentially be used as prebiotic (Ivkovic *et al.*, 2012) has some promising impacts on broilers. Beneficial influence of Actigen® in the current study could be

attributed to its efficacy in maintaining a balanced gut microbial population that might have played a crucial role in improving gut health and performance of the broilers (Gaggia *et al.*, 2010). Improved feed intake, body weight gain and FCR noticed in present study could be related to the suppressive effect of Actigen® on enteric pathogens and better integrity of the intestinal linings of the chickens (Kim *et al.*, 2011; Lea *et al.*, 2011). These authors also reported that dietary *Saccharomyces cerevisiae* cell wall carbohydrate fraction is helpful in reducing stress in birds that assist bird to consume more feed, and coincide with present findings of improved feed intake. Similar results of improved performance were found in a series of experiments with dietary yeast carbohydrate fraction and prebiotics (Safalaoh, 2006). These results are also supported by the work of (Koc *et al.*, 2010) who observed higher feed intake with addition of crude yeast cell wall carbohydrate fraction MOS in poultry diet. In contrast to present findings Midilli *et al.* (2008) did not notice any differences in feed intake, body weight gain with dietary MOS probably due to differences in age and type of birds used. Improved body weight and FCR of birds could be attributed to gut integrity and better nutrient utilization as has been observed in present study. Moreover, villus height and goblet cell counts were significantly improved with Actigen supplementation that probably have resulted in better nutrients assimilation and body weight gain and can be supported by the outcomes of Ivkovic *et al.* (2012). Koc *et al.* (2010) observed similar findings of improved body weight gain when broilers were supplemented with MOS. In another study on broiler during starter phase Yang *et al.* (2007) observed that MOS addition improved body weight gain and feed intake, which support current findings. Findings of better body gain in present study are however in contrast to Midilli *et al.* (2001) who observed no significant effect of dietary supplementation of MOS on body weight gain. Discrepancy in results could probably be the effect of level and type of MOS used and other dietary differences. Birds that received Actigen had better nutrient utilization and body weight gain. Enhanced efficiency of feed utilization could be attributed to the beneficial impact of Actigen on gut health, improved digestion and a reduction in the load of pathogenic microbes that compete for nutrients (Hajati and Rezaei, 2010). Pelicano *et al.* (2004) reported improved FCR in birds that were supplemented MOS from 1 to 21 days and agrees to findings of present study. Similar findings of improved FCR were also reported by Yang *et al.* (2007) and Hooge (2003).

In the current study, an increase villus height and goblet cells count could be attributed to a reduction in the growth of pathogenic intestinal bacteria, binding of anti-nutritional factors in the feed that lowers inflammatory response of intestinal mucosa (Yang *et al.*, 2007) Mannanligosaccharides increases goblet cell counts in the small intestine that in turns enhances mucin production that assists in the abolition of pathogens and provide a fitting environment for digestion and assimilation of nutrients. Increased in goblet cells density in birds supplemented with MOS is consistent with the findings of Uni and Smirnov (2006) and Solis de los Santos *et al.* (2007). These outcomes are supported by the findings of Yang *et al.* (2007) and Maiorka *et al.* (2004)



**Fig. 1:** Photomicrograph of lower ileum of broilers supplemented with different levels of Actigen® indicating villi status at day-21 post hatch. YCF-0 (upper): control (no added Actigen®); YCF-0.8 (middle): 0.8g Actigen® kg-1 diet; YCF-1 (lower): 1g Actigen® kg-1 diet. H&E, ×100.

who did not observe any change in length of intestine, however, reported an improved microstructure villi and goblet cells. Baurhoo *et al.* (2009) noticed a significant increase in intestinal length as opposed to present findings, villus height and goblet cells count. Pelicano *et*

*al.* (2004) detected higher villus length and goblet cell count in ileal region of chicks supplemented with MOS after three weeks of age and support findings of present study.

Dietary prebiotics i.e., MOS can improve the integrity of the intestinal mucosa by binding and inhibiting pathogenic and opportunistic pathogenic bacteria from attaching to the intestinal mucosa thus improve nutrients availability. It also improves the health status of the gastrointestinal tract therefore could be used as indirect growth promoters and for effective nutrients utilization (Patterson *et al.*, 2003). The current study support the findings of Yang *et al.* (2008) who showed that a yeast cell wall MOS fraction enhances the AME of the feed. In contrast, Yang *et al.* (2007) did not find any significant improvement in nutrients and energy availability with MOS supplementation. The improved nutrient digestibility in broilers supplied with yeast cell wall fractions in diets may be due to an improvement in gut health (Hajati and Rezaei, 2010). The effects of oligosaccharides on the digestibility of Ca and P was investigated by (Huang *et al.*, 2005; Li *et al.*, 2007) but these kinds of studies are very limited. The finding of improved digestibility of nutrients is in line with the results of Huang *et al.* (2005) who found that dietary supplementation of oligosaccharides improved nutrient digestibility in broiler chickens.

Teo and Tan (2007) found out the positive effect of prebiotics on the bacterial population in the gastro intestinal tract and observed that birds fed prebiotics have higher lymphoid organs weights to that of the controlled one because prebiotics reduces the pathogenic bacteria in the gut and stimulate the health of the birds. Ahmadi (2011) observed increased in the lymphoid organs weight when the diets were added prebiotics. The former study also observed an increase in the lymphoid organs due to the increase in amount of prebiotics. Brzoska *et al.* (2007) observed significant increase in carcass weight and dressing percentage of birds with prebiotics addition.

**Conclusion:** Present findings revealed that yeast carbohydrate fraction, mannanoligosaccharides (Actigen®, Alltech Inc), supplementation @ 0.5-1.0 g/kg of diet have the potential to improve broiler production performance, gut health and nutrient utilization by broilers during starter phase.

**Author's contribution:** AS and SK developed the research idea and planned the experiment. IU and RU did the field work and collected samples. SK, NAK, HK and RUK analysed the data. All authors approved the manuscript.

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