EFFECTS OF MULTI-ENZYMES ON GROWTH PERFORMANCE, AND EFFECTS OF MULTI-ENZYMES AND PROBIOTICS ON NUTRIENT UTILIZATION IN BROILERS FED DIFFERENT LEVEL OF FIBERS

by

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Abstract

The overall objective was to examine the effect of multi-enzymes on growth performance and that of multi-enzymes and DFM on apparent ileal (AID) and apparent total tract digestibility (ATTD) of nutrients in broilers. Two independent studies were conducted for 21 d with Cobb 500 broilers fed low, and high fiber diets. In growth study, a 2×2 factorial design (2 level fiber and 2 supplements: none or xylanase, amylase, and protease; XAP) with 8 replicate floor pens (8 birds/pen; in two batches) was used. The digestibility study was conducted in a 2×4 factorial design with 8 replicate cages (6 birds/cage; in two batches). Each fiber level diet was supplemented 1) none (control), 2) XAP, 3) DFM or 4) XAP+DFM. In study 1, in 21 d period, the high fiber increased FCR ($P < 0.05$) by 0.04 units compared with low fiber. The XAP supplementation increased ADG by 12% and reduced FCR by 0.09 units compared with the control ($P < 0.01$). In study 2, the high fiber decreased ($P < 0.05$) AID and ATTD of dry matter (DM), crude protein (CP), gross energy (GE) and starch, and ATTD of total NSP and nitrogen corrected apparent metabolizable energy (AMEn). The high fiber decreased ($P <0.01$) AID of all amino acids except AID of lysine, glutamine, phenylalanine and glycine. The combination of XAP and DFM increased ($P <0.01$) total tract NR and AMEn as well as the AID and ATTD of DM, CP, GE, starch and total NSP while the individual supplements had intermediate effects. The combination increased ($P <0.05$) the AID of isoleucine, phenylalanine, threonine, cysteine, and tyrosine. The results suggest that the multi-enzymes can improve the performance of birds raised on fibrous diets while its combination with DFM can produce enhanced improvement in digestibility of nutrients than their single use.

Keywords: AMEn, broiler, direct-fed microbial, performance, XAP

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Chapter 1: Literature review

1.1 Introduction

The production of quality broiler meat at a low per unit cost is a major goal of broiler producers. Feed accounts for almost 70% of the investment, making it worth to formulate a cost-effective diet that would ensure efficient utilization of feed ingredients and optimum production of broilers.

The price of corn and soybean like feed ingredients keeps fluctuating with season and production and the competition between animal and human food causes scarcity of many ingredients. This burden is even more severe due to the use of many agricultural crop products in bio-fuel generation. Recently, there has been an increasing trend of using alternative feedstuffs in addition to the conventional ingredients like corn and soybean meal (SBM) to reduce feed costs. However, high non-starch polysaccharides (NSP) content, an anti-nutritive factor of such feedstuffs like coproducts can decrease nutrient's utilization and slow down growth rate in broilers, thus limit their inclusion. Broiler chickens do not produce endogenous enzymes that would help in NSP digestion; rather they rely on the acidic digestion in the proventriculus and microbial degradation of NSP in large intestine and ceca (Leeson and Summers, 2001). This effect is more prominent in young animals as their GI tract is not matured enough to cope with soluble polysaccharides (Yasar and Forbes, 2000).

The 'cage' theory states that carbohydrases act on cell walls of plant source ingredients, thereby reducing their integrity and release the nutrients that were previously encapsulated. NSP and phytate are not completely disintegrated, so the nutrients entrapped are not properly utilized in poultry (Cowieson et al., 2010). Therefore, the large volume of un-degraded NSP reaches the hind gut of poultry and yield relatively low energy via limited microbial fermentation (Jamroz et

al., 2002). Llikewise some amount of starch and protein from corn soybean-based diet can also escape to the lower intestine (Zanella et al., 1999). The use of individual enzyme has demonstrated a considerable improvement in growth (Gracia et al., 2003; Olukosi et al., 2007; Romero et al., 2013), however, there is a need to improve the digestibility of various types of nutrients in a mixed feed due to the complex nature of feed matrix (Amerah et al., 2017). The xylanase, amylase and protease (XAP) combination increases the AME_n, ileal digestibility of nitrogen and several amino acids (Romero et al., 2013) where protease acts to liberate more AME and digestible amino acids in conjunction with xylanase and amylase by disrupting the starch-protein matrix (Zanella et al., 1999). In addition to releasing nutrient from the feed, XAP reduces intestinal viscosity and improves gut mucosal health in broilers (Gracia et al., 2009).

The incorporation of enzyme combination containing XAP can increase the nutrient digestibility of feed and can thereby enhance average gain (Brunett, 1996; Cowieson and Ravindran, 2008) and improve feed efficiency (Liu et al., 2015) in broilers. The cocktail of XAP also shows an additive effect with phytase by bringing enhancement in body weight gain and improvement in FCR of broilers (Cowieson and Adeola, 2005). There is existing knowledge on the roles of multi-enzymes on growth performance of broilers fed either a variety of cereals and protein sources or a nutrient deficient diet. However, there is a scarcity of data showing the response of multi-enzymes on a diet adequate in energy but varying in fiber content. Even though xylanase, amylase, and protease have yielded improvement in broiler performance, the combination has not shown consistent results when supplemented with corn-soy based diet alone or with added corn DDGS (Cowieson and Adeola, 2005; Olukosi et al., 2010; Zanella et al., 1999). It is well understood fact that only by making a well-balanced and complete diet will not be sufficient to ensure the improved utilization of nutrients by the broiler chicken rather the change

in the population or community of microbes can also affect the gut health and digestion and vice versa.

The term direct fed microbials (DFMs) is interchangeably used for probiotics and these are the source of live micro-organisms that include bacteria, fungi and yeasts as stated in the US National Food Ingredient Association (Miles and Bootwalla, 1991). FAO/WHO has defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). The use of DFM along with multi-enzymes can produce more benefit due to additive or synergistic effect by improving gut health and digestion (Momtazan et al., 2011). Apparently, the outcome of the microbial abundance is complex as there will be high demand of energy and protein by these microbes which will undesirably deprive the host of the essential nutrients contained in the diet (Bedford, 2000). Apart from NSP, another major anti-nutrient in plant-based feed ingredient is a phytic acid which can be hydrolyzed to yield phosphorus using exogenous phytase (Dilger et al., 2004; Woyengo et al., 2010). External phytase is essential as poultry do not possess endogenous enzyme to degrade it (Bedford, 2000). Phytic acid increases the cost of feeding and reduces the utilization of other nutrients besides phosphorus (Lenis and Jongbloed, 1999; Selle and Ravindran, 2007).

The first study focuses on evaluating the effect of XAP on growth performance of broilers fed nutritionally adequate diets varying only in fiber content while containing phytase @ 500 FTU/kg as basal inclusion. and varying only in fiber content. The second study focused on the determination of nutrient utilization in the same diet type as performance study but also evaluated the effect of DFM and their combination with XAP.

1.1.1 Gastrointestinal physiology of broilers

Broiler chicken consumes and store the feed in the crop before passing it to stomach through the lower esophagus. The crop is the diverticulum of the esophagus which also helps in moistening of feed. Broilers do not grind the feed in their mouth as they lack teeth and thus must depend on muscular gizzard for the grinding and mixing of feed. The feed from crop enters the proximal section of the broiler's stomach which is called proventriculus. The proventriculus is the glandular portion and secretes hydrochloric acid, pepsin while the non-glandular muscular gizzard further helps in digestion by breaking feed particles and increasing access of digestive enzymes to substrates. Basically, the digestive process of broiler is like other monogastric animal. The liver secretes bile which helps in emulsification of lipids while pancreas secretes amylase, trypsinogen, pancreatic lipase, chymotrypsinogen, and procarboxypeptidase etc., which are emptied in duodenum and acts on the chyme. The enzymes secreted by the intestine ae maltase, isomaltase, sucrase, enterokinase, lipase and peptidases which contribute to the last step of digestion. The broilers have a pair of ceca which is responsible for fermentation of the residual feed by the action of the resident microbiome. The capacity of broiler ceca may not be as big as in other hind gut fermenters, but it can certainly provide some short chain fatty acids which are either utilized as a source of energy or supports the health mucosal epithelium.

For a sustainable and profitable broiler production, an optimal functionality of the gastrointestinal system is essential. Conway (1994) has proposed three major component of gut health which are the diet, the mucosa, and the commensal microbiome. Certainly, all these components play a critical role in the balance of gut physiology, broiler health, their growth performance, nutrient utilization, and welfare. The classical function of the digestive system is the

digestion of feed by means of enzymatic and microbial fermentation and later absorption of nutrients. The impact of diet on GIT can be directed towards various functions ranging from digestive to immune system. The fibers supplied in the diet can provide prebiotic effect and stimulate beneficial bacteria (Jha and Berrocoso, 2015), but the beneficial effect of these dietary fibers could be offset since high dietary fiber could decrease the total tract digestibility of nutrients (Freire et al., 2000). Hence, feed enzymes can improve animal performance by hydrolyzing the feed substrate that either not broken or broken only partially by the endogenous enzymes, especially in young broilers with relatively immature GIT (Ravindran, 2013). Likewise, a balanced diet, a healthy gut mucosa is of prime importance as it is involved in secretion of enzymes, absorption of nutrients harboring of immune cells. Furthermore, the gut microbiome which interacts with both diet and GIT causes rapid uptake and conversion of metabolic intermediates which is removed from GIT through digestion and absorption by the host and as such creates a highly dynamic system (Celi et al., 2017).

1.1.2 Anti-nutrients in feed ingredients

Anti-nutrients are the class of components found in ingredients that can disrupt the digestion and absorption of nutrients. These components can form insoluble complexes with nutrients like Ca, Zn, Fe etc., and prevent their absorption. Phytic acid and tannins are a type of anti-nutrients that can inhibit enzyme and block normal metabolism of nutrients in feed. The other types of enzyme inhibitor are lectins and trypsin inhibitor which can be found in legumes while another form of antinutrients like NSP increases the viscosity of the feed. Some other toxic molecules like sulfur glycosidase and hydrogen cyanide can severely reduce the value of feed that may contain them.

1.1.3 Non-starch polysaccharides (NSP)

NSP is the major component of plant cell wall, e.g., β-glucans, xylose, and pentosans (Figure 1). The soluble NSP are believed to increase the digesta viscosity in the intestinal tract and decrease the nutrients utilization in broilers (Choct and Annison, 1992). Due to lack of endogenous enzymes to breakdown these NSP, the digestibility of nutrients is reduced, leading to poor performance in broilers (Cowieson and Ravindran, 2008; Meng et al., 2004; Steenfeldt et al., 1998). NSP degrading enzymes can increase metabolizable energy and decrease digesta viscosity (Adeola and Bedford, 2004). In response to increased digesta viscosity, there is an increase in transit time of digesta which in turn brings negative impact on broiler microbiota and growth (Choct and Annison, 1992; Vahjen et al., 1998).

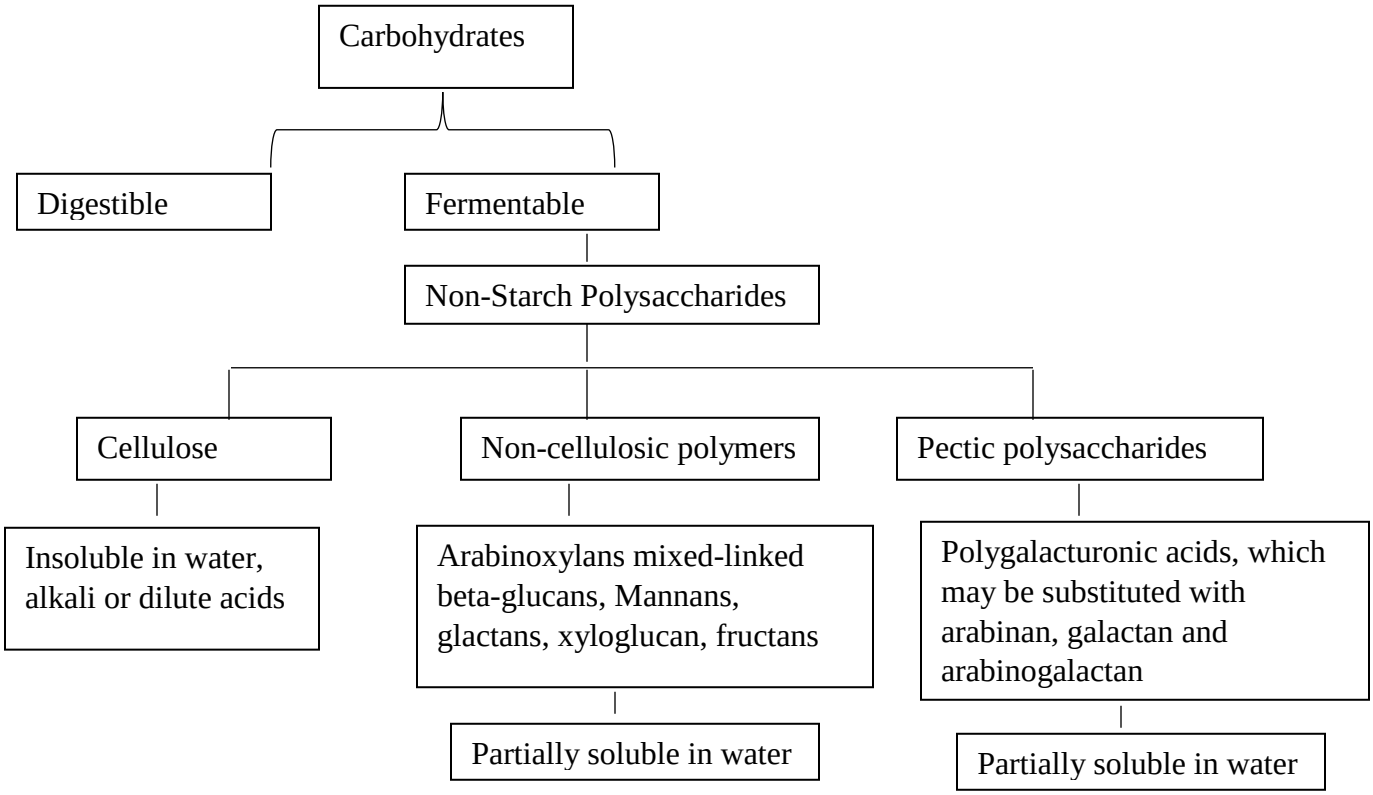

Figure 1 Summary of classification of carbohydrates. Adapted from Choct et al., 2010; Boisen and Verstegen, 2000.

1.1.4 Phytic acid

In plant, phosphorus is present as phytic acid or inositol-6-phosphate (Figure 2). It can bind meal ions such as Zn2+, Ca2+, Cu2+, Fe2+, Mg2+, Mn2+, Mo2+, and Co2+ to form insoluble complexes called phytate. These complexes are not absorbed in GIT. Phytic acid can also bind to some endogenous enzymes like pepsin, trypsin, protease, and lipase and reduce their activity. The unabsorbed phytic acid also leads to an environmental problem as the unutilized P gets washed into surface water and causes eutrophication and destruction of the ecological balance. Phytic acid decreases AME, ileal N and amino acid digestibility (Ravindran et al., 2000). To lower the antinutrient effect of IP6, Adeola and Cowieson (2011) suggested to dephosphorylate IP6 in the GIT as early as possible.

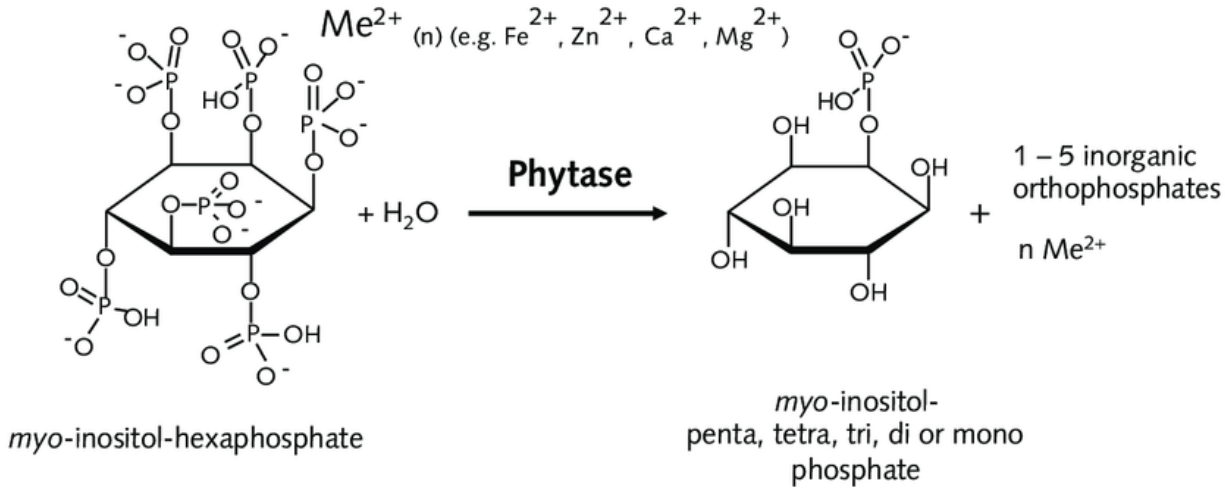

Figure 2 Schematic representation of hydrolysis of phytate (myo-inositol-hexaphosphate) by phytase enzyme. From Troesch et al., 2013.

1.1.5 Exogenous enzymes

Negative effect of several anti-nutrients can be alleviated by grinding, heating, soaking, chelating, or hydrolyzing using enzymes and microbes based on the nature of the component.

Exogenous enzymes that are generally used to enhance nutrient utilization and reduce antinutrient effect are obtained from bacteria or fungi. Exogenous enzymes used in poultry feed are either not produced by the bird or the level of production is not efficient in the host. Xylanase, amylase and protease are being used either individually or in combination to breakdown their substrate and to assist other exogenous or endogenous enzymes by releasing their respective substrates from feed matrix (Figure 3, 4 & 5). Several studies have reported that the addition of carbohydrases to the corn-SBM diets improved the energy utilization by 2-4% (Rutherfurd et al., 2007; Yang et al., 2010). Xylanase supplementation in wheat-based diet or in a diet containing rye, wheat and SBM can improve the broiler performance (Olukosi et al., 2007; Wu et al., 2004). Hydrolysis of both soluble and insoluble NSP decreases water holding capacity of the GIT which results in an accelerated flow of digesta, increased cell wall permeability of plant source ingredients and digestibility of feed (Bedford 1996; Wealleans et al., 2017).

Xylanase can also increase the digestibility and accretion of N by hydrolysis of encapsulating cell wall (Cowieson et al., 2010; Meng and Slominski, 2005). The xylanase addition in the diet of broilers has not always brought positive response (Olukosi et al., 2007; Olukosi and Adeola, 2008). This can be due to differences in the nutrient density reduction of control and supplemented diets (Adeola and Cowieson, 2011). Proper concentration and the combination of NSP enzymes needs to be used to harness optimum benefit as the indiscriminate use of excess of such enzyme may further degrade oligosaccharides in to monosaccharides (Choct et al., 2010; Fuller, 2001) which has potential to cause osmotic diarrhea and deteriorate performance (Schutte, 1990).

Supplementation of carbohydrases has also produced improvement in the digestibility of amino acid in broilers (Bedford et al., 1998; Hew et al., 1998). This could be either due to the

release of proteins bound in the cell wall (Tahir et al., 2008) or due to increase in the availability of glucose which is important in regulating amino acid transporters and protein synthesis pathways (Roos et al., 2009). However, studies on the amino acid digestibility using carbohydrases report conflicting response regarding endogenous amino acid loss (Rutherfurd et al., 2007; Yin et al., 2000).

Exogenous protease and amylase enzymes are often supplemented along with xylanase which not only improves the digestion of protein in the upper GIT of broilers but also increases the digestion of starch in the encapsulated endosperm (Zanella et al., 1999). Exogenous amylase has the tendency to break the bonds in amylopectin compared to endogenous α-amylase (Goesaert et al., 2010). Exogenous protease can complement endogenous peptidase by improving digestibility of protein and by degrading proteinaceous anti-nutrients like lectins or trypsin inhibitors or antigenic proteins in the substrate like SBM (Douglas et al., 2000; Ghazi et al., 2002).

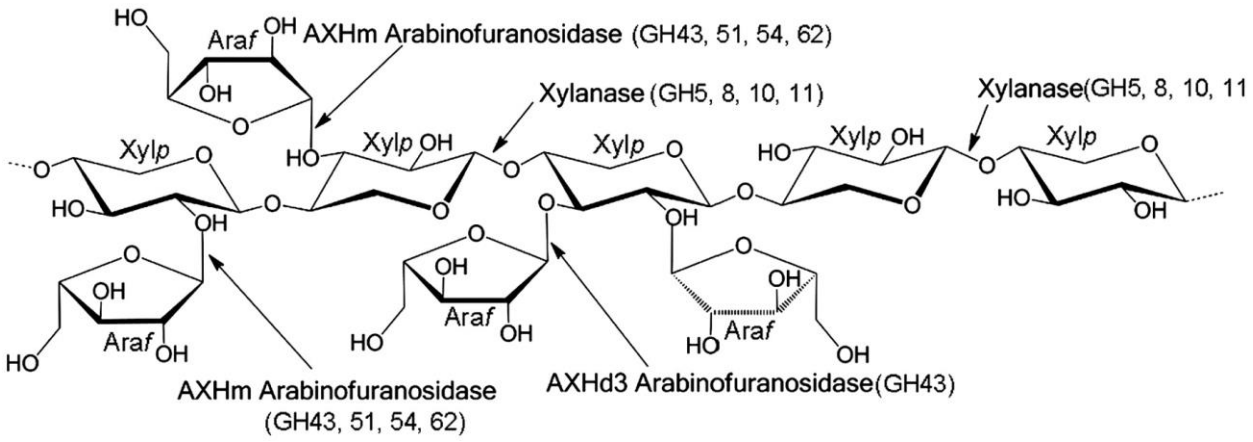

Figure 3 Schematic of different arabinoxylan linkages and the hydrolyzing enzymes. Araf stands for arabinofuranose residues and Xlp stands for xylopyranose units in the xylan backbone. Reproduced from McKee et al., 2012.

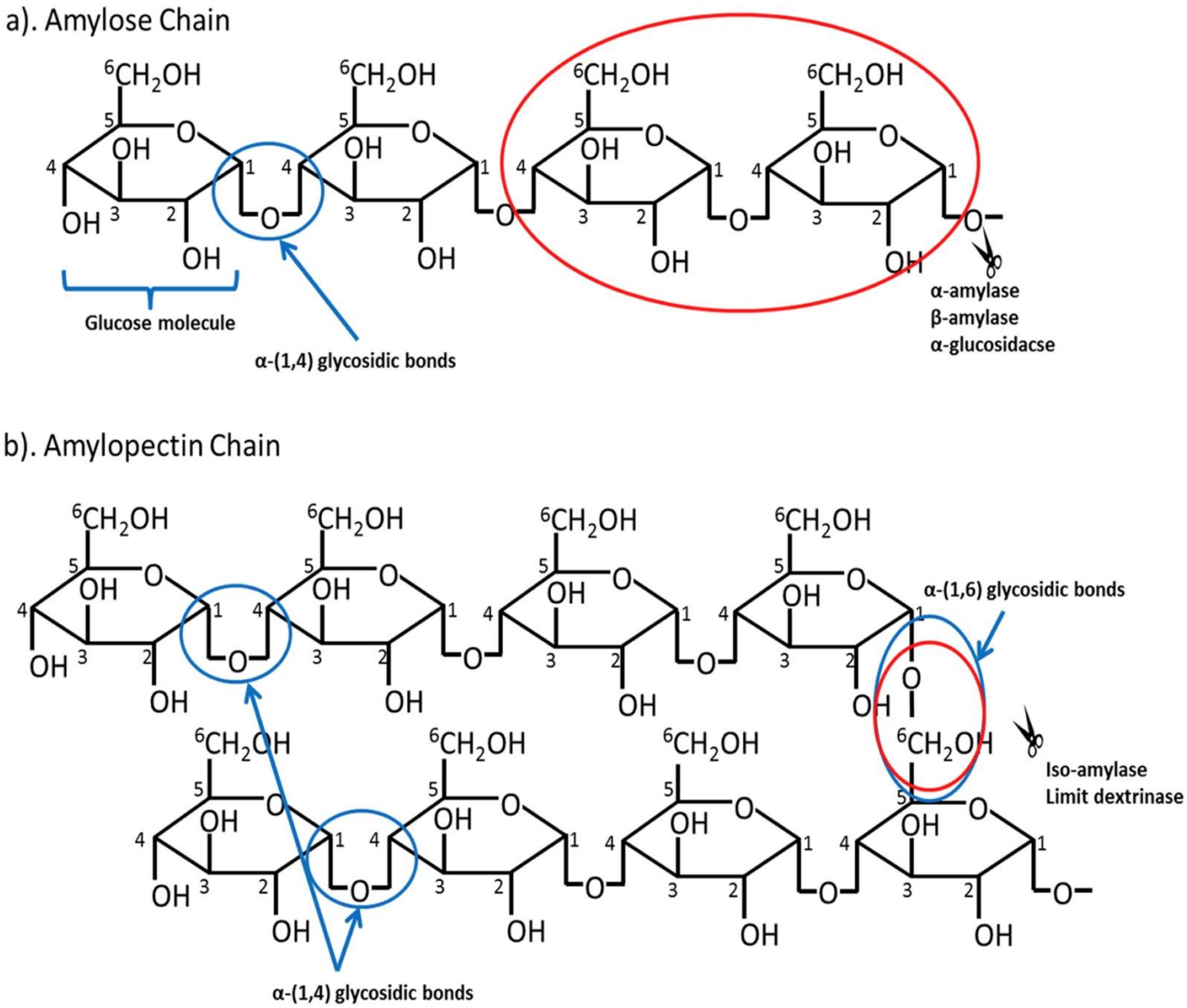

Figure 4 a) Depicts an amylose chain with α-(1 → 4) linkage of glucose molecule which is cut by α-amylase, β-amylase and α-glucosidase to form maltose (glucose + glucose) from the non-reducing end. b) Shows an amylopectin chain made up of chains formed by α-(1 → 4) linkages and branched by α-(1 → 6) linkages which is cut by iso-amylase and limit dextrinase for further degradation by α-amylase, β-amylase. From Gous and Fox, 2017.

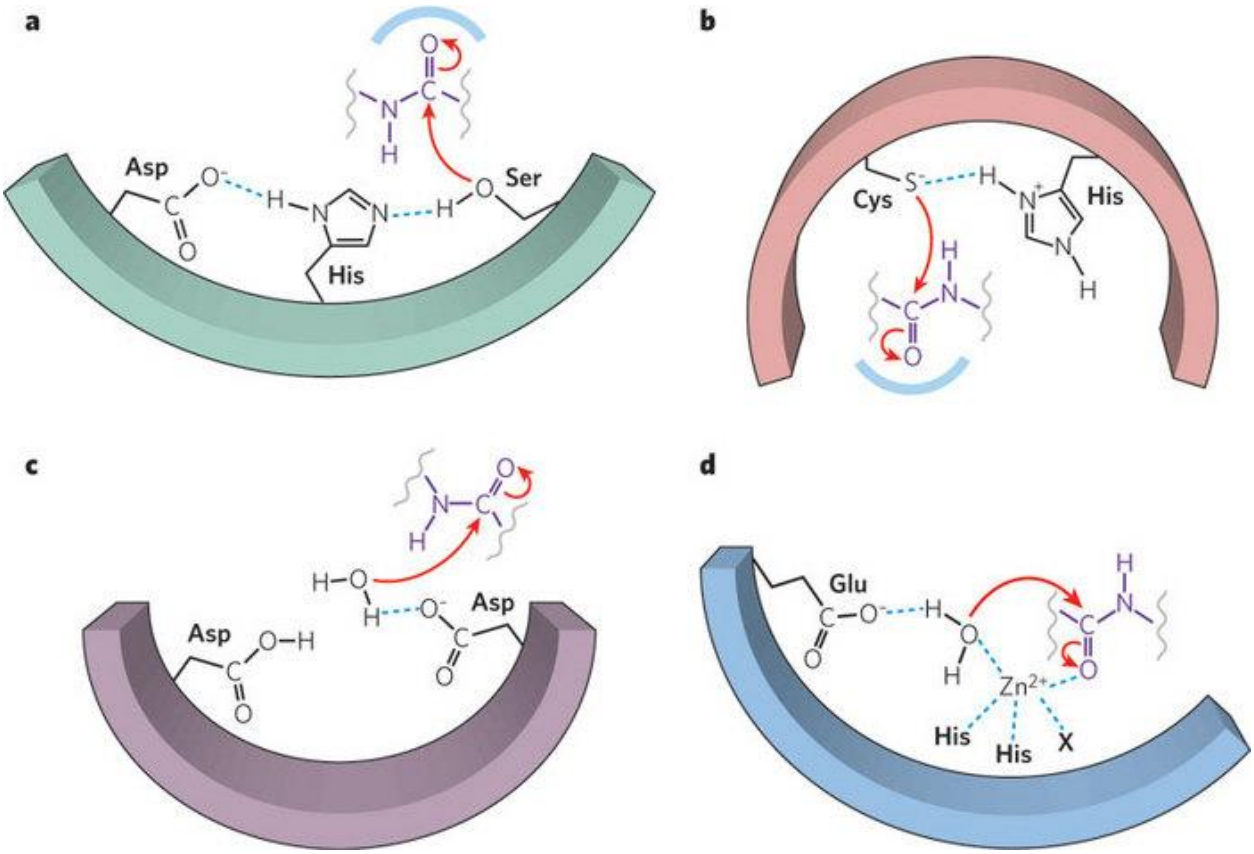

Figure 5 Schematic diagram of action of different classes of protease enzymes. Soluble serine proteases (a); cysteine proteases (b); aspartyl proteases (c); and metalloproteases (d). From Erez et al., 2009.

1.1.6 Probiotics/ direct-fed microbials

Probiotics are defined as live microorganisms that are thought to be beneficial to the host animal by improving their gut microbial balance (Fuller, 1989) or by modifying the properties of the resident microbiota (Havenaar and Huis In'T Veld, 1992). Some examples of probiotics are lactic acid bacteria, bifidobacterial and yeasts. Probiotics are marketed as direct-fed microbials

(DFM) which can play a role in meeting energy requirements of the bird through regulation of fermentation and SCFA synthesis in hind gut (Caballero-Franco et al.,2007).

There is a common practice to limit the incidence of enteric pathological or infectious disease by directly feeding beneficial microbials (DFMs) or probiotics (Choct, 2009). The direct inhibitory effects of DFMs like *Bacillus* strains isolated from poultry litter, swine lagoons, rumen fluids and other environments was evident on avian pathogenic *Escherichia coli* and *Clostridium perfringens* type A i*n vitro* (Rehberger and Jordan-Parrott, 2009). Apart from the direct inhibitory effects of DFMs on the enteric pathogen, they compete with pathogenic microbes for substrates and attachment sites with concurrent production of antimicrobials which help the host in balancing microbial population and enhance immunomodulation (Yang et al., 2009). In addition to those primary activities, colonic microflora produces short chain fatty acids (SCFA) which is partially responsible for circulation and bowel motility suggesting a form of established mutualism (Kvietys and Granger, 1981). Probiotics can also modulate either innate or adaptive or both immune system (Dugas et al., 1999). The DFM provides benefit through regulation of microbial homeostasis and maintenance of barrier function in the GIT (Salminen et al., 1996). The DFM supplied via feed can also regulate the hind-gut fermentation and synthesis of short-chain fatty acids and thus, increase the efficiency of the gut microbes to ferment NSP (Sakata, 1987).

Ahmed et al. (2014) reported that *Bacillus amyloliquefaciens* probiotic used as direct-fed microbial (DFM) increased average daily feed intake (ADFI), increased averaged daily gain (ADG) but decreased feed conversion ratio (FCR) in an overall experimental period of 35 days. In the same experiment, the DFM inclusion modulated the immunity by increasing the level of serum IgG and IgA. Meng et al. (2010) reported a positive effect of *Bacillus subtilis* containing

probiotics combination on total tract digestibility of energy and protein, and growth performance of growing-finishing pigs fed low nutrient (energy, protein, and lysine) density diet. Several strains of *Bacillus subtilis* can produce amylase, protease, pectinase and glucanase enzymes in *in vitro* study (Hmani et al., 2017). Inclusion of DFM in broiler diet can promote uptake of glucose, amino acid and minerals across the intestinal epithelium by maintaining the mucosal structural and functional integrity (Wu, 1998; Li et al., 2008).

1.1.7 Summary

In summary, this literature review presented an outline of the negative effect of NSPs and phytate on growth performance and nutrient utilization in broilers. This review managed to precisely present the results from different studies on the effect of exogenous enzyme and direct fed microbials or probiotic on growth performance and nutrient utilization. Presence of anti-nutritional factors in the corn-SBM diet as well pose a challenge to the economic production of broilers. While some anti-nutrients can be eliminated through processing of raw materials, others like NSP and phytate can be targeted through the supplementation of exogenous carbohydrase and phytase enzymes. Overall, a positive response of exogenous enzyme combination has been noted on broilers growth performance and nutrient utilization. Likewise, supplementation of DFM constructively modulates gut microbiota, gut barrier function, competitively excludes pathogenic bacteria and produces enzymes *in situ*. The cocktail of xylanase, amylase and protease can provide benefit by improving digestibility where xylanase targets hemicellulose in cell wall and releases entrapped starch and protein while amylase and protease assist by hydrolyzing these substrates. In addition to assisting in gut health, DFM could help in digestion

of nutrients by releasing enzymes and boosting endogenous enzymes. Hence, supplementation of a XAP and DFM can increase bird's performance, feed efficiency, and nutrient digestibility. The aim of this experiment was to figure out the nature of the possible interaction between the levels of fibers and types of supplements and to discern the phenomenon like antagonism, additive or synergistic effects that occur in the combination.

1.1.8 Hypotheses

For the first study in which multi-enzymes was used to test the growth performance, we hypothesized that the supplementation of XAP would be more effective in improving feed efficiency and growth of broiler chicken fed fibrous diet during the early growth period of 0 -21 d. In the second study, multi-enzymes and DFM were incorporated in diets to determine the nutrient's digestibility. For the second study, we hypothesized that the supplementation of XAP and DFM would be complementary to each other and their combination would produce a more pronounced improvement in the digestibility of energy, protein and other nutrients in broiler chicken fed different levels of fiber.

1.1.9 Objectives

The objectives of both studies were set to test the respective experimental hypothesis about the effects of supplemental multi-enzymes or the combination of multi-enzymes and DFM on broiler's performance and feed utilization. The objective of study 1 was to determine the effects of fiber and XAP on ADFI, ADG and FCR of young broilers during 0-21 d of age. The objective of study 2 was to determine the effects of fiber, XAP, DFM and the combination of

XAP and DFM on the apparent ileal and the apparent total tract digestibility of nutrients in 21 d old broilers.

Chapter 2: Materials and methods

Study 1: Effects of a combination of xylanase, amylase, and protease on growth performance of broilers fed low and high fiber diets

2.1 Growth performance

2.1.1 Experimental design and diets

The animal study protocol was carried out in accordance with the Institutional Animal Care and Use Committee of the University of Hawaii, USA. A total of 256 day-old broiler chicks (Cobb × Cobb 500) were reared at the University of Hawaii at Manoa (Honolulu, HI, USA) in floor pens housed in environmentally controlled rooms. The broilers received 12 h of high and 12 h of low fluorescent illumination and were provided with ad libitum feed and water. The chicks were weighed individually, wing tagged and placed randomly in one of the 16 pens (eight birds per pen) in 2 batches, making 8 replicates for each treatment.

In each pen (experimental unit), the birds were provided with one of the four dietary treatments. The diets were formulated (Table 1) to contain the same amount of metabolizable energy and protein level across the treatments and mineral and vitamins were supplemented to meet or exceed the nutrient requirements of broilers (NRC, 1994). The dietary treatments contained 2 levels of fiber (low and high) and 2 applications of XAP (without or with supplementation) in a 2 × 2 factorial arrangements in a completely randomized design. The XAP (Danisco Animal Nutrition/Dupont, Marlborough, UK) supplied 2000 U of xylanase, 200 U of

amylase and 4000 U of protease per kg of diet. All the diets contained 500 FTU/kg of phytase and were pelleted. The enzyme activity recovery in feed for xylanase (76–130%), amylase (71–94%), and protease (88–107%) was analyzed and was meeting the targeted levels in the XAP supplemented feed, considering the large variation in feed mixing and lab analysis. The level of fiber was increased in high fiber diet by the inclusion of wheat middlings, canola meal, and corn DDGS. The feed consumption and the body weight of the birds was measured weekly at same time. Any leftover feed in the feeder were weighed and recorded weekly for calculating the net feed intake, which was further corrected for mortality on intake/day basis. The data generated were used to calculate ADFI, ADG, and FCR.

2.1.2 Chemical analysis

The feed samples were analyzed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2006) with specific methods as follows: dry matter (DM) was calculated by placing in hot air oven at 65 °C for overnight (method 930.15). The gross energy (GE) was determined using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL, USA). The nitrogen (N) content was determined using the Leco method (method 990.03) and was used to calculate the crude protein (CP) content (N× 6.25). Ether extract was determined by Soxhlet method (method 920.39), and the ash content was measured by burning the samples at 650 °C for overnight (method 942.05). Acid detergent fiber (ADF, method 973.18) and neutral detergent fiber (NDF, method 2002.04) was determined using Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA).

2.1.3 Statistical analysis

All the data sets were analyzed using MIXED procedure of SAS (SAS v 9.2, SAS Institute, Cary, NC, USA) to compare test variables where diets were the fixed factor and batch was a random factor. The test model included the main effects of fiber level and XAP along with their interaction. The means were separated using Tukey's method and the difference was considered significant at $P \leq 0.05$ and considered a trend at $P < 0.10$.

Table 1 Ingredient composition and nutritive value of the experimental diets in study 1(g/kg, as-fed basis unless otherwise indicated).

	Low fiber diet		High fiber diet	
Ingredient	**Control**	**XAP***	**Control**	**XAP***
Corn	614.9	614.9	389.3	389.3
Wheat	-	-	70.0	70.0
Wheat middlings	-	-	70.0	70.0
Soybean meal	332.7	332.7	236.6	236.6
Canola meal	-	-	60.0	60.0
Corn DDGS	-	-	90.0	90.0
Meat and bone meal	24.0	24.0	22.8	22.8
Soya oil	5.0	5.0	38.7	38.7
Limestone	4.9	4.9	5.6	5.6
Dicalcium phosphate	2.2	2.2	-	-
Lysine	1.2	1.2	2.5	2.5
Methionine	2.0	2.0	1.8	1.8
Threonine	0.0	0.0	0.3	0.3
Salt	3.1	3.1	2.4	2.4
Vitamin mix[1]	5.0	5.0	5.0	5.0
Mineral mix[2]	5.0	5.0	5.0	5.0
Phytase	0.1	0.1	0.1	0.1
Calculated analysis				
Metabolizable energy (MJ/Kg)	12.43	12.43	12.48	12.48
Crude protein	227.0	227.0	229.0	229.0
Calcium	5.8	5.8	5.9	5.9
Total phosphorus	5.5	5.5	5.9	5.9
Non-phytate phosphorus	2.8	2.8	2.7	2.7
Lysine	12.8	12.8	12.9	12.9
Methionine	5.4	5.4	5.3	5.3
Threronine	8.2	8.2	8.3	8.3
Methionine+ Cystine	9.1	9.1	9.2	9.2
Crude fiber	27.2	27.2	41.2	41.2
Sodium	3.1	3.1	2.7	2.7
Chloride	2.4	2.4	2.1	2.1
Choline	3.9	3.9	3.4	3.4
Analyzed value				
Dry matter	889.0	887.0	886.0	896.0
Gross energy (MJ/kg)	16.55	16.55	16.69	16.69
Ash	42.3	42.0	42.6	42.5
Crude protein	212.1	214.1	214.3	215.0
Ether extract	29.3	29.5	66.8	66.9
NDF	72.3	73.2	113.2	111.6
ADF	46.0	47.5	53.2	54.5

*XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet.

[1]Supplied per kilogram of diet: 66138 IU of vitamin A, 19841 IU of vitamin D_3, 331 IU of vitamin E, 0.2 mg of vitamin B_{12}, 1.3 mg of biotin, 19.8 mg of vitamin K_3, 19.8 mg of thiamine, 66 mg of riboflavin, 110 mg of d-pantothenic acid, 40 mg of pyridoxine, 551 mg of niacin and 11 mg of folic acid.

[2]Supplied per kilogram of diet: 300 mg of manganese, 300 mg of zinc, 200 mg of iron, 25 mg of copper, 6.25 mg of iodine and 2.5 mg of cobalt.

Study 2: Effects of a combination of xylanase, amylase and protease (XAP), and DFM on nutrients digestibility in broilers fed low and high fiber diets

2.2 Digestibility of nutrients

2.2.1 Animals and housing

A total of 384 day-old broiler chicks (Cobb 500) were used in the digestibility study. Considering the housing capacity of the small animal facility, the study was conducted over 2 consecutive periods, each having an equal number of birds in each treatment and raised in similar environment. In each period, 192 day-old chicks were weighed individually, wing tagged and placed randomly in each cage with 6 birds, making 4 replicates of each treatment in each period. The chicks were housed in the environmentally controlled room and brooding temperature was maintained with supplemental heat. The temperature ranged from 35^0C in the first week to around 25^0C attained at the end of the 3rd week by gradual reduction. The chicks were provided photoperiod of 24-hour light with ad libitum and unrestricted access to feed and water at all the times. The birds were also monitored at least in the morning and evening daily, and all incurring mortalities were recorded along with the feed intake till that date.

2.2.2 Diets and experimental design

The diets were prepared maintaining the same caloric value and protein level across the treatments (Table 2). The diets were formulated with supplementation of mineral and vitamins to meet or exceed the nutrients requirements of broiler (NRC, 1994). The treatments contained 2 levels of fibers and 4 combinations of additives in a 2×4 factorial arrangements. The two fiber levels were low and high fiber and supplements were a) control, without supplementation, b) multi-enzymes (XAP), c) DFM, and d) the combination of multi-enzymes and DFM (XAP +DFM). All the diet contained 500 FTU/kg of phytase and was pelleted for 3 weeks feeding. Birds in each cage were fed with one of 8 diets where diet was the treatment, and each cage birds were the experimental unit. The multi-enzymes supplied 2000 U of xylanase, 200 U of amylase and 4000 U of protease per kg of diet. The DFMs contained 3 *Bacillus spps* strains and were included at the rate of 75,000 CFU per kg of diet. The level of fiber was increased in high fiber diet by the inclusion of wheat midds, canola meal and corn DDGS. The multi-enzymes and DFMs were supplied by Danisco Animal Nutrition/ Dupont, UK and was applied as top dressing on the feed.

2.2.3 Sample collection

Excreta samples were collected per cage over three consecutive days (from day 18 to 20) for the determination of AME and was store at -20 ^{0}C until further analysis. The collected excreta samples were pooled and mixed in a blender and later lyophilized, ground and stored in airtight containers at room temperature. On day 21, all the birds were euthanized by intracardial injection of sodium pentobarbitone (> 100 mg/kg body weight) and the contents of the distal ileum were expelled out by gentle milking in order to avoid mucosal sloughing. The digesta

sample was immediately snap frozen in dry ice to avoid microbial degradation and later stored at -20 ^{0}C until further analysis.

2.2.4 Chemical analysis

The samples of feed, ileal digesta and fecal contents were analyzed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2006) with specific methods as follows: DM by placing in hot air oven at 65^{0}C for overnight (method 930.15). The samples for other nutrients analysis were dried by lyophilization. The gross energy was determined by using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL) with benzoic acid as a calibration standard. The nitrogen content was ascertained using Leco method (method 990.03) and used to calculate CP content (N×6.25). Ether extract was determined by Soxhlet method (method 920.39) and the ash content was determined by burning sample at 650^{0}C for overnight (method 942.05). The ADF (method 973.18) and NDF (method 2002.04) were analyzed using Ankom fiber analyzer. Total starch content was analyzed using commercial kit (Megazyme International, Ireland). Chromic oxide was determined by perchloric acid method (Fenton and Fenton, 1979). Complete amino acid profile was obtained by using HPLC (method 982.30E a,b,c; AOAC, 2006). Total NSP was determined on feed, ileal digesta and excreta by quantifying their constituent neutral sugars by gas chromatography. Uronic acid was not determined in the present study. Total neutral sugars were quantified as described by Englyst et al. (1994). Chromatographic analysis was done using a GC system (TRACETM 1300 gas chromatograph, Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector and a fused silica capillary column (DB-17HT, Agilent Technologies, Wilmington, DE, USA), using 2-Deoxy-D-Glucose as an internal standard. The

AME was determined by marker method using equation below as performed by Robbins and Firman (2006) on ileal digesta.

$AME = GE_{diet} \times [(1-Cr_2O_{3\ diet} / Cr_2O_{3\ excreta}) \times (GE_{excreta} / GE_{diet})]$

Or, $AME = GE_{diet} - [GE_{excreta} \times Cr_2O_{3\ diet} / Cr_2O_{3\ excreta}]$

Both apparent ileal and total tract digestibility of individual nutrients were calculated using following equation:

$AID\% = 1- [(Cr_2O_{3\ diet} / Cr_2O_{3\ digesta}) \times (Nut.\ Conc_{digesta} / Nut.\ Conc_{diet})]$
$ATTD\% = 1- [(Cr_2O_{3\ diet} / Cr_2O_{3\ excreta}) \times (Nut.\ Conc_{excreta} / Nut.\ Conc_{diet})]$

[Cr_2O_3= chromic oxide, Nut.Conc= Nutrient concentration]

2.2.5 Statistical analysis

The data obtained were analyzed using MIXED procedure of SAS (SAS v9.2, SAS Institute, Cary, N.C., USA) to compare test variables where diets were fixed factor and batch was a random factor. The interaction between fiber levels and inclusion of additive were tested. Finally, the means were separated using Tukey's method and significance was declared at $P<0.05$.

Table 2 Ingredient composition and nutritive value of the experimental diets in study 2 (g/kg, as-fed basis unless otherwise indicated).

Ingredients	**Low fiber diet**				**High fiber diet**			
	Control	XAP[#]	DFM[*]	XAP+DFM	Control	XAP[#]	DFM[*]	XAP+DFM
Corn	63.92	63.92	63.92	63.92	53.05	53.05	53.05	53.05
Wheat midds	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.00
SBM	29.93	29.93	29.93	29.93	19.90	19.90	19.90	19.90
Canola meal	0.00	0.00	0.00	0.00	4.25	4.25	4.25	4.25
Corn DDGS	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.00
Meat and bone meal	2.00	2.00	2.00	2.00	3.88	3.88	3.88	3.88
Soya oil	0.50	0.50	0.50	0.50	2.00	2.00	2.00	2.00
Limestone	1.09	1.09	1.09	1.09	0.92	0.92	0.92	0.92
Di-cal Phosphate	0.65	0.65	0.65	0.65	0.00	0.00	0.00	0.00
Lysine	0.00	0.00	0.00	0.00	0.15	0.15	0.15	0.15
Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Threonine	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08
Tryptophan	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02
Nacl	0.34	0.34	0.34	0.34	0.23	0.23	0.23	0.23
Vitamin mix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Chromic oxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated nutrient content								
AMEn, (kcal/kg)	2962	2962	2962	2962	2935	2935	2935	2935
Crude protein %	21.06	21.06	21.06	21.06	21.01	21.01	21.01	21.01
Crude fiber %	2.64	2.64	2.64	2.64	3.42	3.42	3.42	3.42
Lysine %	1.08	1.08	1.08	1.08	1.10	1.10	1.10	1.10
Methionine %	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55
Threonine %	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80
Methionine + Cysteine %	0.89	0.89	0.89	0.89	0.90	0.90	0.90	0.90
Ca %	0.84	0.84	0.84	0.84	0.87	0.87	0.87	0.87
Total P %	0.61	0.61	0.61	0.61	0.60	0.60	0.60	0.60
Na %	0.32	0.32	0.32	0.32	0.34	0.34	0.34	0.34
Cl %	0.26	0.26	0.26	0.26	0.28	0.28	0.28	0.28
Choline (mg/kg)	3952	3952	3952	3952	3760	3760	3760	3760
Determined nutrient content (%)								
Dry matter	88.64	87.82	88.59	88.51	88.53	88.51	88.92	89.08

GE, kcal/kg	4001	3970	4000	3995	4090	4090	4111	4115
Crude protein	20.97	20.78	20.99	20.92	20.63	20.63	20.62	20.68
Total ash	5.01	5.05	5.21	5.03	4.99	5.07	4.93	5.07
Starch	34.02	34.56	34.72	34.97	31.32	31.25	31.05	31.65
Ether extract	3.11	3.15	3.07	3.15	5.34	5.24	5.31	5.29
Total NSP[3]	9.25	9.19	9.23	9.15	11.03	11.05	11.03	11.07

>Phytase was added @ 500FTU/kg in all diets

[#]XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. [*]DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g)

[1]Supplied per kilogram of diet: 66138 IU of vitamin A, 19841 IU of vitamin D_3, 331 IU of vitamin E, 0.2 mg of vitamin B_{12}, 1.3 mg of biotin, 19.8 mg of vitamin K_3, 19.8 mg of thiamine, 66 mg of riboflavin, 110 mg of d-pantothenic acid, 40 mg of pyridoxine, 551 mg of niacin and 11 mg of folic acid.

[2]Supplied per kilogram of diet: 300 mg of manganese, 300 mg of zinc, 200 mg of iron, 25 mg of copper, 6.25 mg of iodine and 2.5 mg of cobalt.

[3]Total NSP does not contain uronic acid. Only total neutral sugar determined using gas chromatography was considered for this study.

Chapter 3: Results

Study 1: Effects of a combination of xylanase, amylase, and protease on growth performance of broilers fed low and high fiber diets

3.1 Growth performance

The nutrient compositions of low and high fiber diets were similar in terms of CP, essential amino acids, vitamins and minerals (Table 1). The calculated AME content of high fiber diets was slightly higher than low fiber diets due to the addition of extra oil. The ADFI, ADG and FCR of the birds were calculated for each week and for total period (Table 3). A significant interaction ($P < 0.05$) between fiber level and XAP was found for ADFI in the overall study period (d 0-21). The enzyme supplementation increased ADFI more in low fiber group than in high fiber fed birds. Additionally, significant ($P < 0.05$) main effect was observed where low fiber group had lower ADG but was accompanied by improved FCR by 0.04 unit compared with the high fiber group. The XAP supplementation increased ADFI by 4.7%, ADG by 12% and consequently lowered FCR by 0.09 units relative to the un-supplemented diets. Specifically, the XAP supplementation improved ($P < 0.01$) ADFI in low fiber diets and reduced FCR in high fiber diet while increased ADG in both groups.

In the first week, the high fiber diet decreased ADG by 6.3% with an associated increase in FCR by 0.08 units ($P < 0.05$). Across two fiber diets, XAP increased ADG by 15.8% and decreased FCR by 0.15 units ($P < 0.01$). The ADFI remained unaffected by fiber or XAP; however, an interaction ($P < 0.01$) between fiber level and XAP was found for ADG and FCR. In the high fiber group, XAP increased ADG by 24.5% while in low fiber group it improved ADG by a lesser amount of 7.7%. XAP reduced FCR in high fiber fed group by 0.27 units, but it decreased FCR

only by 0.05 units in low fiber group. In the second week (d 8-14), high fiber increased ADFI by 9.1%, ADG by 3.2%, but it was associated with poor FCR increased by of 0.07 units ($P < 0.05$). The XAP supplementation had no effect on ADFI ($P > 0.05$), but it increased ADG by 7.3% and improved FCR by correspondingly reducing it by 0.09 units ($P < 0.01$). Unlike in the first week, no significant interaction ($P > 0.05$) was observed in the second week between fiber level and XAP with respect to ADG and FCR.

In the third week, a tendency ($P = 0.0982$) was observed on FCR in regard to fiber level. The XAP supplementation improved ($P < 0.01$) FCR by 0.11 units compared to the control group. Except for FCR, a significant interaction ($P < 0.05$) was observed between fiber level and XAP on ADFI and ADG. The XAP increased ADFI by 15.3% and ADG by 21.8% in low fiber group while it increased ADFI by 2.8% and ADG by 10.9% in high fiber group.

Table 3 Growth performance of broiler chickens from 1 to 21 days of age when fed two levels of fibers without or with XAP.

Variable	Main effects					Treatments[3]					P- value		
	Fiber		Supplements			Low Fiber		High Fiber					
	Low	High	Control	XAP	SEM[1] (n=16)	Control	XAP	Control	XAP	SEM[2](n=8)	Fiber	Supplements	Fiber × Supplements
ADFI (g/day)													
Day 0-7	19.6	19.6	19.5	19.7	0.85	19.4	19.8	19.7	19.5	0.94	1.000	0.509	0.659
Day 8-14	46.7^{b}	51.0^{a}	49.1	48.9	1.95	46.3	47.0	51.2	50.8	2.13	0.001	0.589	0.400
Day 15-21	92.2^{b}	99.2^{a}	92.3^{b}	99.2^{a}	3.42	84.8^{b}	97.8^{a}	97.9^{a}	100.6^{a}	3.74	0.001	0.001	0.010
Day 0-21	53.0^{b}	56.4^{a}	53.5^{b}	56.0^{a}	1.55	50.3^{b}	55.0^{a}	55.9^{a}	57.0^{a}	1.70	<.001	0.002	0.027
ADG (g/day)													
Day 0-7	17.6^{a}	16.5^{b}	15.8^{b}	18.3^{a}	0.57	16.9^{a}	18.2^{a}	14.7^{b}	18.3^{a}	0.65	0.019	<.001	0.010
Day 8-14	39.8^{b}	41.1^{a}	39.0^{b}	41.8^{a}	1.45	38.3	41.0	39.5	42.7	1.59	0.042	0.002	0.851
Day 15-21	61.0^{b}	63.7^{a}	57.7^{b}	66.5^{a}	0.86	54.2^{c}	66.0^{a}	60.4^{b}	67.0^{a}	1.21	0.004	<.001	0.037
Day 0-21	39.2^{b}	40.5^{a}	37.5^{b}	42.0^{a}	1.00	36.1	41.5	38.5	42.5	1.12	0.022	<.001	0.308
FCR													
Day 0-7	1.11^{b}	1.19^{a}	1.23^{a}	1.08^{b}	0.06	1.14^{b}	1.09^{b}	1.34^{a}	1.07^{b}	0.06	0.038	<.001	0.010
Day 8-14	1.17^{b}	1.24^{a}	1.26^{a}	1.17^{b}	0.04	1.21	1.15	1.30	1.19	0.04	0.022	0.005	0.290
Day 15-21	1.51	1.56	1.60^{a}	1.49^{b}	0.05	1.57	1.48	1.62	1.50	0.05	0.098	0.008	0.332
Day 0-21	1.35^{b}	1.39^{a}	1.43^{a}	1.33^{b}	0.03	1.39	1.33	1.45	1.34	0.04	0.027	0.001	0.202

[a-c] Within rows in the respective group of factors (main effects-fiber & XAP and Treatments), means without a common superscript differ ($P < 0.05$). XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet

[1]Pooled SEM (n= 16 replicate cages containing 8 birds per cage)

[2]Pooled SEM (n= 8 replicate cages containing 8 birds per cage)

[3]Means separation in treatments shows a significant interaction effect

Study 2: Effects of a combination of xylanase, amylase and protease (XAP), and DFM on nutrients digestibility in broilers fed low and high fiber diets

3.2 Digestibility of nutrients

The apparent ileal digestibility of total ash, DM, GE, CP, EE, starch, total NSP and AIDE was determined from ileal digesta collected from 21 d old broiler (Table 4). There was no interaction between fiber and supplements for AID. Neither fiber type nor supplement or their combination had any significant effect on AID of ash and ether extract ($P > 0.05$). High fiber decreased AID of DM, GE, CP and starch ($P < 0.05$), but did not affect total NSP ($P > 0.05$). The treatment with combined activities of XAP and DFM improved the ileal digestibility of DM, GE, CP, starch and total NSP compared with the control ($P < 0.001$). The single activity of XAP and DFM also improved ($P < 0.001$) the ileal digestibility of DM and CP while XAP only increased the ileal digestibility of GE and starch.

No interaction was observed between fiber and supplements for ATTD, i.e., nutrient retention (Table 5). As in case of ileal digestibility, ATTD of total ash and EE was not affected by any treatments ($P > 0.05$). High fiber decreased the ATTD of DM, GE, CP, starch, total NSP, nitrogen retention along with reduction in AMEn ($P < 0.01$). The combined activities of XAP and DFM improved the ATTD of DM, GE, CP, starch, total NSP, NR and increased AMEn compared with the control ($P < 0.01$). Although numerically higher effects of XAP and DFM were observed on ATTD of CP and NR, the effects were not significant ($P > 0.05$) compared with the control and was neither significantly ($P > 0.05$) lower than the combination. The XAP group had a numerically better ATTD compared with DFM for GE, starch, total NSP and AMEn while

the effect was not significantly ($P > 0.05$) different than the combination of XAP and DFM but was an intermediate magnitude.

An interaction ($P < 0.05$) was only observed for the total mannose in the ileal flow of total NSP components analyzed for neutral sugars (Table 6). High fiber increased ($P < 0.01$) the magnitude of total NSP and total neutral sugars components that included arabinose, xylose and glucose while rhamnose, fucose and galactose were not affected. The ileal flow of total fucose, rhamnose, xylose and glucose were not affected by supplements ($P > 0.05$). Administration of XAP and DFM alone did not reduce the ileal flow of total NSP compared with the control. However, the combination of XAP and DFM significantly ($P < 0.001$) reduced the ileal flow of total arabinose, galactose and total NSP compared with the control. There was higher reduction in the magnitude of ileal flow of total mannose by the XAP and DFM combination in low fiber group compared with high fiber group. The total tract flow of total neutral sugars and total NSP was a mirror effect of ileal flow of NSP except that the magnitude of effect was greater for total NSP in total tract flow, rhamnose was affected by both fiber and supplements and there was no significant interaction ($P > 0.05$) between fiber and supplements for total mannose (Table 7). High fiber increased ($P < 0.01$) the total tract flow of total rhamnose, arabinose, xylose, mannose, glucose and total NSP. The combination of XAP and DFM reduced ($P < 0.001$) the total tract flow of total arabinose, mannose, galactose and total NSP compared with the control group while the improvement by XAP and DFM was intermediate. The supplements also decreased the flow of rhamnose ($P < 0.05$), the effect that was not evident for the ileal flow.

Table 4 Effects of multienzymes and DFM on apparent ileal digestibility (AID) of nutrients in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

		Variables							
Treatments		Total Ash, %	DM, %	GE, %	CP, %	EE, %	Starch, %	Total NSP, %	AIDE, kcal/kg
Low Fiber	Control	38.2	70.3	70.0	73.4	86.0	96.0	21.2	3160
	XAP	41.9	74.4	71.5	75.8	87.0	97.1	25.0	3233
	DFM	39.4	73.6	70.9	74.8	86.7	96.7	23.4	3199
	XAP+DFM	43.3	76.1	72.4	77.1	87.6	97.4	27.9	3267
High fiber	Control	36.8	69.9	67.8	71.1	85.2	95.8	20.9	3132
	XAP	39.4	72.7	69.1	72.9	86.1	96.4	23.6	3195
	DFM	37.2	71.6	68.4	72.2	85.8	96.0	22.4	3163
	XAP+DFM	41.1	74.6	69.7	73.8	86.7	96.9	25.6	3221
	SEM[1] (n=8)	2.96	1.39	0.52	0.42	1.68	0.37	1.27	23.74
Main effects (Factors)									
Fiber	Low	40.7	73.6^{a}	71.2^{a}	75.3^{a}	86.8	96.8^{a}	24.4	3215^{a}
	High	38.6	72.2^{b}	68.8^{b}	72.5^{b}	86.0	96.3^{b}	23.1	3178^{b}
	SEM (n=32)	2.34	1.22	0.26	0.25	0.84	0.32	0.63	11.87
Supplements	Control	37.5	70.1^{c}	68.9^{c}	72.2^{c}	85.6	95.9^{c}	21.0^{b}	3146^{c}
	XAP	40.6	73.5^{ab}	70.3^{ab}	74.4^{b}	86.5	96.7^{ab}	24.3^{ab}	3214^{ab}
	DFM	38.3	72.6^{b}	69.6^{bc}	73.5^{b}	86.3	96.4^{bc}	22.9^{b}	3181^{bc}
	XAP+DFM	42.2	75.3^{a}	71.0^{a}	75.4^{a}	87.1	97.1^{a}	26.8^{a}	3244^{a}
	SEM (n=16)	2.57	1.28	0.37	0.31	1.19	0.34	0.90	16.79
P- value	Fiber	0.159	0.014	<0.001	<0.001	0.477	0.002	0.173	0.030
	Supplements	0.110	<0.001	0.001	<0.001	0.837	<0.001	<0.001	<0.001
	Fiber× Supplements	0.994	0.743	0.979	0.596	1.000	0.675	0.870	0.986

[a-c] Within a column, means without a common superscript differ (P < 0.05)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

Table 5 Effects of multienzymes and DFM on apparent total tract digestibility (ATTD) of nutrients in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

	Variables									
Treatments		Total Ash, %	DM, %	GE, %	CP, %	EE, %	Starch, %	Total NSP, %	NR, g/kg	AMEn, kcal/kg
Low Fiber	Control	29.9	76.6	79.5	68.9	86.5	96.3	33.1	26.1	3373
	XAP	32.5	78.8	80.3	70.0	87.7	97.2	37.4	26.5	3413
	DFM	31.4	77.4	80.1	69.6	87.1	96.8	35.8	26.4	3400
	XAP+DFM	34.6	80.7	81.0	70.9	88.0	97.2	39.7	26.8	3437
High fiber	Control	29.2	74.1	76.6	64.8	85.8	95.9	31.9	24.2	3340
	XAP	31.4	76.6	77.3	65.6	86.5	96.3	35.1	24.5	3372
	DFM	31.0	75.9	77.1	65.5	86.2	96.2	33.6	24.3	3365
	XAP+DFM	33.1	77.7	77.9	66.4	87.1	96.6	36.9	24.7	3396
	SEM[1] (n=8)	2.64	0.53	0.36	0.51	1.66	0.31	0.94	0.19	15.40
Main effects (Factors)										
Fiber	Low	32.1	78.4^{a}	80.2^{a}	69.8^{a}	87.3	96.9^{a}	36.5^{a}	26.4^{a}	3406^{a}
	High	31.2	76.1^{b}	77.2^{b}	65.6^{b}	86.4	96.3^{b}	34.4^{b}	24.4^{b}	3368^{b}
	SEM (n=32)	2.22	0.37	0.26	0.37	0.98	0.24	0.50	0.14	10.75
Supplements	Control	29.6	75.4^{c}	78.0^{c}	66.8^{b}	86.1	96.1^{b}	32.5^{c}	25.1^{b}	3357^{c}
	XAP	32.0	77.7^{b}	78.8^{ab}	67.8^{ab}	87.1	96.7^{a}	36.2^{ab}	25.5^{ab}	3393^{ab}
	DFM	31.2	76.6^{b}	78.6^{bc}	67.6^{ab}	86.6	96.5^{ab}	34.7^{bc}	25.3^{ab}	3382^{bc}
	XAP+DFM	33.9	79.2^{a}	79.5^{a}	68.6^{a}	87.5	96.9^{a}	38.3^{a}	25.7^{a}	3417^{a}
	SEM (n=16)	2.37	0.43	0.29	0.42	1.25	0.27	0.68	0.16	12.49
P- value	Fiber	0.435	<0.001	<0.001	<0.001	0.414	<0.001	0.002	<0.001	<0.001
	Supplements	0.084	<0.001	<0.001	<0.001	0.817	0.003	<0.001	0.002	<0.001
	Fiber× Supplements	0.986	0.353	0.972	0.945	0.999	0.783	0.876	0.869	0.983

[a-c] Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

Table 6 Effects of multienzymes and DFM on ileal flow (g/kg DM) of components of total non-starch polysaccharides in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

		Variables							
Treatments		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Glucose	Galactose	Total NSP
Low Fiber	Control	1.74	2.33	17.96	16.77	2.97^{b}	24.06	16.46	82.3
	XAP	1.72	2.31	16.35	16.69	2.15^{cd}	23.56	15.72	78.5
	DFM	1.73	2.33	16.68	16.78	2.38^{c}	23.86	16.08	79.8
	XAP+DFM	1.68	2.26	14.87	16.06	1.82^{d}	23.28	14.50	74.5
High fiber	Control	1.79	2.34	21.36	22.43	3.41^{a}	30.52	16.70	98.6
	XAP	1.75	2.30	19.98	22.37	3.01^{b}	30.03	15.86	95.3
	DFM	1.79	2.31	20.27	22.39	3.11^{ab}	30.34	16.06	96.3
	XAP+DFM	1.74	2.27	19.01	22.09	2.85^{b}	29.96	14.55	92.5
	SEM[1](n=8)	0.07	0.07	0.41	0.67	0.09	0.57	0.73	1.44
Main effects (Factors)									
Fiber	Low	1.72	2.31	16.46^{b}	16.58^{b}	2.33	23.69^{b}	15.69	78.8^{b}
	High	1.77	2.31	20.16^{a}	22.32^{a}	3.09	30.21^{a}	15.79	95.7^{a}
	SEM (n=32)	0.05	0.04	0.21	0.36	0.04	0.38	0.64	0.72
Supplements	Control	1.76	2.34	19.66^{a}	19.60	3.19	27.29	16.58^{a}	90.4^{a}
	XAP	1.74	2.31	18.16^{b}	19.53	2.58	26.80	15.79^{a}	86.9^{ab}
	DFM	1.76	2.32	18.48^{b}	19.59	2.74	27.10	16.07^{a}	88.1^{a}
	XAP+DFM	1.71	2.26	16.94^{c}	19.08	2.34	26.62	14.53^{b}	83.5^{b}
	SEM (n=16)	0.06	0.05	0.29	0.49	0.06	0.46	0.67	1.02
P value	Fiber	0.123	0.986	<0.001	<0.001	<0.001	<0.001	0.718	<0.001
	Supplements	0.615	0.796	<0.001	0.833	<0.001	0.519	<0.001	<0.001
	Fiber× Supplements	0.993	0.996	0.829	0.988	0.012	0.995	0.990	0.931

[a-d] Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

Table 7 Effects of multienzymes and DFM on total tract flow (g/kg DM) of components of total non-starch polysaccharides in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

		Variables							
Treatments		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Glucose	Galactose	Total NSP
Low Fiber	Control	1.54	1.62	15.20	16.80	2.27	23.80	8.55	69.8
	XAP	1.48	1.58	13.52	16.62	1.60	23.23	7.46	65.5
	DFM	1.51	1.61	13.97	16.74	1.82	23.63	7.63	66.9
	XAP+DFM	1.45	1.55	12.30	15.99	1.32	23.05	6.69	62.4
High fiber	Control	1.59	1.61	17.57	22.67	2.37	30.03	9.06	84.9
	XAP	1.57	1.59	16.03	22.43	1.83	29.62	7.95	81.0
	DFM	1.59	1.60	16.55	22.59	2.04	29.82	8.15	82.3
	XAP+DFM	1.51	1.58	15.45	21.80	1.69	29.36	7.00	78.4
	SEM[1] (n=8)	0.05	0.03	0.35	0.45	0.06	0.63	0.37	1.11
Main effects (Factors)									
Fiber	Low	1.50^{b}	1.59	13.75^{b}	16.54^{b}	1.75^{b}	23.43^{b}	7.58	66.1^{b}
	High	1.56^{a}	1.59	16.40^{a}	22.37^{a}	1.98^{a}	29.71^{a}	8.04	81.7^{a}
	SEM (n=32)	0.04	0.02	0.18	0.27	0.04	0.48	0.23	0.62
Supplements	Control	1.56^{a}	1.62	16.39^{a}	19.73	2.32^{a}	26.92	8.80^{a}	77.3^{a}
	XAP	1.53^{ab}	1.59	14.78^{bc}	19.53	1.72^{c}	26.42	7.71^{bc}	73.3^{b}
	DFM	1.55^{ab}	1.60	15.26^{b}	19.67	1.93^{b}	26.73	7.89^{b}	74.6^{ab}
	XAP+DFM	1.48^{b}	1.56	13.88^{c}	18.90	1.51^{d}	26.21	6.85^{c}	70.4^{c}
	SEM (n=16)	0.04	0.03	0.25	0.34	0.05	0.53	0.28	0.82
P value	Fiber	0.001	0.866	<0.001	<0.001	<0.001	<0.001	0.054	<0.001
	Supplements	0.032	0.357	<0.001	0.170	<0.001	0.449	<0.001	<0.001
	Fiber× Supplements	0.884	0.846	0.706	1.000	0.095	0.996	0.988	0.977

[a-c] Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

An interaction ($P < 0.05$) between Fiber and Supplements was observed for AID of histidine, Methionine and Tryptophan among indispensable amino acids but not for dispensable amino acids (Table 8 & Table 9). High fiber decreased ($P < 0.01$) the AID of arginine, isoleucine, leucine, threonine, valine, alanine, asparagine, cysteine, serine, and tyrosine. The AID of lysine and glutamine was not affected ($P > 0.05$) by the fiber level while there was a trend ($P = 0.052$) for phenylalanine and glycine. The combination of XAP and DFM improved ($P < 0.05$) the AID of isoleucine, phenylalanine, threonine, cysteine, and tyrosine while the individual use of XAP and DFM only produced intermediate improvement. The improvement in the AID of lysine and methionine was not significant ($P > 0.05$). The treatment group having incorporation of both XAP and DFM had an improved ($P < 0.05$) AID of amino acids in average while the individual inclusion of either XAP or DFM only generated intermediate effect which was not statistically significant ($P > 0.05$) with either the control or the combination.

Table 8 Treatment effects of multienzymes and DFM on apparent ileal digestibility (AID) of amino acids in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

	Treatments											
	Low fiber				High fiber					P value		
Variables	Control	XAP	DFM	XAP+DFM	Control	XAP	DFM	XAP+DFM	SEM[1] (n=8)	Fiber	Supplements	Fiber× Supplements
CP	73.4	75.8	74.8	77.1	71.1	72.9	72.2	73.8				
Indispensable AA												
Arg	76.7	76.3	75.6	77.9	70.8	72.9	69.2	72.9	1.24	<0.001	0.098	0.655
His	72.3[b]	81.9[a]	80.3[a]	81.0[a]	75.8[ab]	75.9[ab]	71.2[b]	79.7[a]	1.41	0.002	<0.001	<0.001
Ile	75.3	81.1	77.9	81.9	75.9	74.6	73.4	79.1	1.35	0.001	0.002	0.070
Leu	74.6	76.1	77.2	78.7	71.1	71.1	75.0	79.1	1.44	<0.001	0.119	0.290
Lys	75.6	77.8	74.5	78.4	74.7	75.1	75.4	76.6	1.40	0.268	0.228	0.604
Met	79.9[ab]	78.6[ab]	78.9[ab]	80.9[a]	77.0[b]	80.4[ab]	78.7[ab]	77.4[ab]	0.80	0.042	0.605	0.005
Phe	74.1	78.9	78.2	78.9	73.9	76.3	74.6	77.3	1.42	0.052	0.026	0.653
Thr	75.7	76.5	78.1	80.8	71.8	74.4	75.1	77.3	1.57	0.007	0.012	0.948
Trp	78.1[ab]	75.0[ab]	73.3[b]	76.9[ab]	75.1[ab]	78.2[ab]	79.9[a]	78.5[ab]	1.43	0.041	0.820	0.014
Val	75.6	80.6	78.3	79.9	74.4	76.3	73.8	75.7	1.19	<0.001	0.019	0.476
Dispensable AA												
Ala	76.9	79.5	80.8	79.3	72.1	75.6	75.7	75.8	1.19	<0.001	0.013	0.900
Asp	74.4	75.0	77.8	78.3	67.8	73.5	70.6	71.0	1.44	<0.001	0.056	0.149
Cys	74.9	76.4	72.1	78.6	70.9	71.5	69.6	73.0	0.82	<.0001	<.0001	0.276
Glu	76.4	76.8	75.2	76.0	72.8	75.2	76.8	73.2	1.40	0.107	0.571	0.272
Gly	76.3	77.4	76.8	77.0	75.4	74.8	74.8	75.8	1.33	0.081	0.973	0.926
Ser	76.7	78.8	79.7	78.7	72.4	76.4	74.8	76.4	1.24	<0.001	0.050	0.650
Tyr	78.0	75.6	78.1	80.4	72.5	72.3	74.8	75.8	1.28	<0.001	0.016	0.771
Average	76.0	77.8	77.2	79.0	73.2	75.0	74.3	76.2	0.99	<0.001	0.040	0.988

[a-b] Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

Table 9 Main effects of fiber and supplements (XAP, DFM) on apparent ileal digestibility (AID) of amino acids in study 2 in broilers fed low and high level of fibers (DM basis; 0-21 d post-hatch).

	Main effects (Factors)								P -value		
	Fiber				Supplements						
Variables	Low	High	SEM [1](n=32)	Control	XAP	DFM	XAP+DFM	SEM[2] (n=16)	Fiber	Supplements	Fiber× Supplements
CP	75.3	72.5		72.2	74.4	73.5	75.4				
Indispensable AA											
Arg	76.6[a]	71.5[b]	0.62	73.7	74.6	72.4	75.4	0.87	<0.001	0.098	0.655
His	78.9	75.6	0.70	74.1	78.9	75.7	80.3	1.00	0.002	<0.001	<0.001
Ile	79.0[a]	75.8[b]	0.68	75.6[b]	77.8[ab]	75.7[b]	80.5[a]	0.96	0.001	0.002	0.070
Leu	76.7[a]	74.1[b]	0.72	72.9	73.6	76.1	78.9	1.02	<0.001	0.119	0.290
Lys	76.6	75.5	0.70	75.2	76.5	74.9	77.5	0.99	0.268	0.228	0.604
Met	79.6	78.4	0.40	78.5	79.5	78.8	79.2	0.57	0.042	0.605	0.005
Phe	77.5	75.5	0.71	74.0[b]	77.6[ab]	76.4[ab]	78.1[a]	1.01	0.052	0.026	0.653
Thr	77.8[a]	74.7[b]	0.79	73.8[b]	75.5[ab]	76.6[ab]	79.1[a]	1.11	0.007	0.012	0.948
Trp	75.8	77.9	0.72	76.6	76.6	76.6	77.7	1.01	0.041	0.820	0.014
Val	78.6[a]	75.0[b]	0.60	75.0[b]	78.5[a]	76.1[ab]	77.8[ab]	0.84	<0.001	0.019	0.476
Dispensable AA											
Ala	79.1[a]	74.8[b]	0.59	74.5[b]	77.6[ab]	78.2[a]	77.6[ab]	0.84	<0.001	0.013	0.900
Asp	76.4[a]	70.8[b]	0.72	71.1	74.3	74.2	74.7	1.02	<0.001	0.056	0.149
Cys	75.5[a]	71.2[b]	0.41	72.9[bc]	73.9[ab]	70.9[c]	75.8[a]	0.58	<0.001	<0.001	0.276
Glu	76.1	74.5	0.70	74.6	76.0	76.0	74.6	0.99	0.107	0.571	0.272
Gly	76.9	75.2	0.67	75.9	76.1	75.8	76.4	0.94	0.081	0.973	0.926
Ser	78.4[a]	75.0[b]	0.62	74.5	77.6	77.3	77.5	0.88	<0.001	0.050	0.650
Tyr	78.0[a]	73.9[b]	0.64	75.2[ab]	74.0[b]	76.4[ab]	78.1[a]	0.91	<0.001	0.016	0.771
Average	77.5[a]	74.7[b]	0.50	74.6[b]	76.4[ab]	75.8[ab]	77.6[a]	0.70	<0.001	0.040	0.988

[a-c] Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (32 cage of 8 birds in each treatment).

[2]Pooled SEM (16 cage of 8 birds in each treatment).

>XAP was supplemented (top-dressed) @ 100 gm per MT of diet. XAP is a combination of xylanase, Amylase and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. DFM was added @ 100 gm per MT and contained DFM-3 Bacillus spp. strains (75000CFU/g).

Chapter 4: Discussion

The objectives of these studies were to determine the changes in digestibility of feed and growth pattern of broilers fed different level of fibers in feed supplied without or with multi-enzymes or combination of multi-enzymes and probiotics.

Study 1: Effects of a combination of xylanase, amylase and protease on growth performance of broilers fed low and high fiber diets

4.1 Growth performance

The negative impact of increasing fiber in the broiler diet was evident from reduced feed utilization by high fiber fed broilers during the early period compared to the performance of low fiber fed birds of same ages. Young broiler chicks do not have well developed GIT and can be sensitive to the inclusion of high level of fiber in the diet and thus have shown a poor gain in the 1st week. Despite both high and low fiber diets were formulated with a similar level of AME, high fiber group had higher ADFI and higher ADG but had poor FCR. The higher amount of NSP present in the high fiber diet would have decreased the digestibility of nutrients including starch, protein, fats and minerals (Choct et al., 1996). The increased ADFI in high fiber group would either be due to the better palatability of high fiber diet having higher oil inclusion or due to thermostatic control in broilers. The carbohydrate generates more latent heat of digestion compared to fat due to the requirement of active transport and hence the high fiber group having more oil would have lesser thermogenesis than low fiber group (Ferket and Gernat, 2006).

When the performances of both low and high fiber groups were analyzed for the impact of supplemental XAP, ADFI were similar in all groups without or with XAP until the second week after which the XAP supplemented groups exhibited increment in ADFI in low fiber groups.

Unlike the trend of ADFI, ADG was increased and FCR was reduced by the supplemental XAP in each week and in the overall study period. This improvement in growth performance of broiler chickens by the supplemental XAP is in agreement with the study of Wu et al. (2004) where xylanase was used in a wheat-based broiler diet. Similar improvement in growth performance using xylanase was observed in broilers fed wheat and rye-based diets (Olukosi et al., 2007). The increment of feed intake by XAP in overall study period is in consensus with Cowieson and Ravindran (2008) when broilers were fed XAP supplementation. This increase in ADFI was also reported by Gracia et al. (2003) when protease and amylase were supplemented in broiler diet. The ADG increased in overall study period by XAP supplementation is similar but more than that observed by Zanella et al. (1999) and Cowieson and Ravindran (2008) while it is close to the body weight improvement reported by Cowieson and Adeola (2005) in broilers supplemented with XAP. Besides weight gain, improvement in FCR of broilers by supplemental XAP was also similar to that reported by Cowieson and Ravindran (2008) and Amerah et al. (2017).

Regarding the influence of XAP supplementation at different level of fibers, the overall ADFI was increased in low fiber groups and ADG was improved in both levels of fiber. The improvement in the FCR of high fiber by XAP was comparable to the FCR of low fiber controls which is in agreement with the performance results obtained by Rexen (1981). In the first week, a significant interaction between fiber and XAP was evident with above 20% reduction in FCR in high fiber groups supplemented with XAP. Although no significant interaction was found, XAP reduced FCR ($P< 0.05$) by 0.11 and 0.12 units in high fiber diets in week 2 and 3 respectively. In low fiber group, XAP supplementation numerically reduced FCR by 0.05, 0.06 and 0.09 units in week 1, 2 and 3, respectively. These results indicate that XAP is more effective in improving feed efficiency in high fiber diets, especially in young birds. The improvement in

growth performance is based on the underlying improvement in digestibility of the feed ingredients by exogenous enzymes which is not easily tapped by the endogenous enzymes of the host. The nutrients from corn can be efficiently extracted if the diet is supplemented with exogenous enzymes (Romero et al., 2014) but the degree of response may vary with addition of other substrates (Jha et al., 2015). The amount of exogenous enzymes required to be incorporated in a feed either in matrix application or in top-dressing will depend on the type and characteristics of other ingredients used in that feed. These exogenous enzymes can help in digestion of NSP in the small intestine and later these NSP, either broken or intact would enter the more anaerobic environment in the hind-gut. Once this intact or hydrolyzed NSP reach the hind gut, it will support the population of microbes that will ferment these substrates and will produce the metabolites (Jha et al. 2010), which in turn will boost the health and overall growth performance of broilers. Thus, the hypothesis that XAP brings improvement in growth and feed conversion in broilers fed high fiber diet is supported during the early starter period. Moreover, XAP can generate improvement in feed efficiency in both low and high fiber diet even after the early starter period.

4.2 Digestibility of nutrients

The main substrate for the action of xylanase enzyme in the cell wall is the hemicellulose arabinoxylans (Chesson, 2001). The inclusion of exogenous xylanase in the broiler diet can break the highly branched arabinoxylan of the cell wall and provide access to the endogenous and exogenous enzymes to breakdown protein and starch in their endosperm (Bedford and Cowieson, 2012; Cowieson, 2005). Soluble NSP, especially arabinoxylans present in wheat-

based diet are only marginally degraded in the GIT and increases the viscosity of the digesta which causes a decline in the overall rate of digestion (Annison and Choct, 1991; Bedford, 2000). Improvement in AMEn and growth performance in both wheat and corn-based diet by addition of xylanase indicates hydrolysis of both soluble and insoluble NSP (Kiarie et al., 2014). The inclusion of high fiber from corn-DDGS and canola meal in corn-soy based diet reduces both ileal and total tract digestibility of energy (Bell, 1993; Salim et al., 2010). In the present study, the ileal total NSP was not affected by the level of fiber included in the diet and thus suggest that xylanase enzyme would have acted based on the availability of the substrate. The reduction in the digestibility of protein and starch due to the presence of high level of fiber in both ileal and total tract level suggests that viscosity of digesta and encapsulation of nutrients would make direct access of protease and amylase difficult for the hydrolysis of appropriate substrates. The amylase used in this experiment would be expected to have directly targeted the starch degradation while the protease could have increased the accessibility of amylase by releasing starches encased in protein matrix (Svihus et al., 2005). The digestibility of fat was not affected either by fiber level or by supplements and this could be due to the presence of different level of fat in both low and high fiber diets, most of which was added in feed and would not need enzymes to release it from the cell. However, the lack of improvement in the digestibility of fat agrees with Amerah et al. (2017) and Romero et al. (2014) as the authors did not observe any increment in fat digestibility due to the addition of XA and XAP in corn-soy diet.

The absence of interaction between fiber and supplements indicates that additional improvement in high fiber diets due to supplements did not take place and that may be due to either small difference in fiber level in the two dietary groups or due to the need of different level of enzyme activity to tap the benefits. To optimize the dose of exogenous enzymes, both the targeted

ingredients and their feed matrix has to be examined with the use of different multi-enzymes combination (Pack and Bedford, 1998). In a 21-day broiler study by Adebiyi and Olukosi (2015), there was no wheat-DDGS×XAP interaction for DM retention, AME or AMEn. The authors found that there was a linear decrease in DM retention and AME by wheat DDGS while the supplemental XAP tended to increase DM retention and AME. High level of fiber in the diet achieved through incorporation of wheat-DDGS in wheat-SBM diet reduced DM and energy retention in broilers (Bolarinwa and Adeola, 2012). Similarly, the addition of fiber through corn-DDGS in a corn-SBM diet reduced energy retention and AME in comparison to the basal corn-SBM diet (Adeola and Ileleji, 2009; Adeola et al., 2010). In the current study, the total NSP digestibility in ileum is not affected by the fiber level but only by supplements while there is increase in total tract digestibility of total NSP in low fiber group. This may be due to enhanced activity of hind-gut microbes to use up the low fiber substrate more rapidly while in the ileum, the enzymes may not have been able to cause breakdown the NSP to any further extent. The improvement in CP digestibility by addition of XAP could be an extra improvement due to the addition of protease (Amerah et al., 2017; Romero et al., 2013). The relative improvement in the digestible energy was higher in ileal level compared to total tract level which was also reported by Amerah et al. (2017) and, Cowieson and Ravindran (2008) and can be attributed to less availability of substrate in the hind-gut when XAP enzymes are used in the diet.

The DFMs containing three strain of *Bacillus spp.* were used in this study for their better performance in terms of shelf life, durability during processing and viability throughout distribution and use (Cartman et al., 2008). It is expected that the proper enzymes and DFMs combination can increase the digestibility of energy, proteins and decrease the activity of

pathogenic bacteria by making the easily fermentable substrate more accessible to the beneficial bacteria (Bedford and Cowieson, 2012). Probiotics can interact with other gut bacteria and they are principally used to stabilize gut colony and improve gut health (Hong et al., 2005). Moreover, due to their ability to produce some enzymes like protease and amylase, they can also contribute in increasing digestibility in the host and lead to their improved growth performance (Hmani et al., 2017). The combination of DFM and XAP multi-enzymes led to additional improvement in the ileal and total tract digestibility of nutrients than their individual application. This effect shows that a beneficial/synergistic interaction between enzyme and DFM takes place for improving nutrients digestibility. It has been inferred from the available researches that xylanase enzyme produces prebiotic effect by breaking the carbohydrates into simpler oligosaccharides (Bedford and Cowieson, 2012). In the present study, the combination of XAP and DFM increased the nutrient digestibility in both ileal and total tract, reduced ileal and total tract total NSP flow and improved the digestibility of some indispensable and dispensable amino acids compared to control. Multiple enzymes like xylanase, amylase, glucanase, phytase, protease, pectinase and mannanase, etc., and some other additives like DFM, organic acids are generally used to enhance the digestibility and utilization of nutrients in broiler feeds, but their mechanism of action and efficacy may depend upon their combination and the types of substrate (Cowieson, 2010).

4.3 Conclusion and recommendations

The results indicate that XAP supplementation improved the average daily gain in both groups, irrespective of the low or high level of fibers. The high fiber content decreased feed efficiency in broilers, especially in the first week of age. Supplementation of XAP enzymes

improved feed efficiency more effectively with a high level of fiber during young age. Thus, the combination of exogenous XAP can be helpful in improving body weight gain and feed efficiency in broiler chickens fed diets with cost-effective co-products containing high fiber contents.

High fiber in the co-product decreases the digestibility of gross energy, crude protein, starch, total NSP and amino acids but with the supplementation of XAP and DFM, the digestibility coefficient of various nutrients are improved and effectively optimized even in high fiber feedstuffs. The combination of XAP and DFM has produced more improvement in the ileal as well as total tract digestibility of several nutrients which suggest that there is a synergistic association between exogenous enzymes and DFM.

Even if there was improvement in both low and high fiber diets by the incorporation of XAP and DFM, the improvement in high fiber was not as much as expected, especially regarding the digestibility of nutrients. This may have occurred due to several reasons ranging from variability in response of fibers added from different sources like wheat, canola meal and corn DDGS or due to proteolytic degradation of xylanase enzyme when high digesta viscosity in high fiber diet decreased the passage rate of the intestinal digesta. This study had a limitation that weekly performance record of broilers was not documented due to the nature of the research as the birds needed to be starved for 8-12 hours before feeding all at the same time for 4 hours on 21 d for ileal sample collection. In future, for such studies, digestibility sample can be collected next day which would not make a major difference in nutrient utilization. Also, it would be valuable to gather information on enzyme activity in digesta as well if feasible so that the interaction of enzyme with the specific matrix of different level of fiber could be interpreted in further detail. In the present study, a narrow difference was kept in the level of fiber between low and high

fiber diet in order to avoid major antinutrient effect but in future, the difference can be increased, and fiber can be included at various percentage to determine whether the effect of enzyme is linear or various degree polynomial. Further research on the understanding of synergism, antagonism and passage rate of multi-enzymes along with the microbiota and villi characteristics can provide a clear picture of the requirement and appropriate combination of enzyme and DFM for maximum nutrient utilization by the host.

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Appendix

Table 10 Proximate analysis of Feed, ileal digesta, excreta from study 2 (DM basis; values in percentage unless otherwise stated)

			Variables							
Item	**Fiber**	**Supplement**	Total ash	OM	GE, kcal/kg	CP	EE	Starch	Total NSP	Chromic oxide
Feed	**Low**	Control	5.65	94.35	4514	23.65	3.51	38.38	10.44	0.21
		XAP	5.75	94.25	4521	23.66	3.58	39.35	10.47	0.21
		DFM	5.88	94.12	4515	23.69	3.46	39.20	10.42	0.21
		XAP+DFM	5.69	94.31	4514	23.64	3.56	39.50	10.33	0.21
	High	Control	5.64	94.36	4620	23.31	6.03	35.38	12.46	0.26
		XAP	5.73	94.27	4622	23.31	5.92	35.31	12.48	0.26
		DFM	5.55	94.45	4623	23.19	5.98	34.92	12.41	0.26
		XAP+DFM	5.70	94.30	4620	23.22	5.94	35.54	12.43	0.25
Ileal digesta	**Low**	Control	11.71	88.29	4581	21.24	1.68	5.21	28.05	0.72
		XAP	13.12	86.88	5079	22.55	1.79	4.56	31.31	0.84
		DFM	13.59	86.41	5057	22.93	1.76	4.89	30.97	0.81
		XAP+DFM	13.86	86.14	5372	23.35	1.91	4.41	32.43	0.90
	High	Control	11.89	88.11	5018	22.70	3.01	4.97	33.17	0.88
		XAP	12.25	87.75	5042	22.32	2.91	4.39	33.62	0.92
		DFM	12.05	87.95	5062	22.35	2.94	4.81	33.32	0.89
		XAP+DFM	12.41	87.59	5172	22.50	2.91	4.12	34.18	0.94
Excreta	**Low**	Control	17.99	82.01	4217	33.51	2.17	6.44	31.77	0.96
		XAP	19.36	80.64	4456	35.50	2.25	5.60	32.76	1.06
		DFM	19.17	80.83	4268	34.23	2.12	5.92	31.80	1.00
		XAP+DFM	20.14	79.86	4648	37.38	2.31	5.91	33.84	1.13
	High	Control	16.29	83.71	4414	33.51	3.52	5.87	34.67	1.06
		XAP	16.98	83.02	4550	34.69	3.46	5.63	35.14	1.12
		DFM	16.52	83.48	4592	34.73	3.56	5.83	35.64	1.12
		XAP+DFM	17.09	82.91	4591	35.07	3.42	5.37	35.21	1.14