CANADIAN PEARL MILLET: A POTENTIAL ALTERNATIVE GRAIN TO CORN IN BROILER PRODUCTION

by

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ABSTRACT

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Canadian Pearl Millet: a potential alternative grain to corn in broiler diets

Two studies were conducted to investigate the effects of partially or totally replacing corn with pearl millet (PM) in broiler diets, alone or in combination with exogenous enzymes, on growth performance, ileal dry matter (DM), CP and amino acid digestibility, and intestinal digesta viscosity, morphological development and microbial populations. In experiment 1, dietary treatments included: a standard corn-soybean meal diet and one in which corn was replaced with 25, 50, 75 or 100% PM. In experiment 2, dietary treatments were: 1) a standard corn-soybean meal diet; 2) a PM-soybean meal diet; 3) diet 1 + exogenous enzymes; and 4) diet 2 + exogenous enzymes. All diets were formulated to be isonitrogenous and isocaloric. PM diets contained less soybean meal because PM grains were richer in CP and amino acids than corn. Total replacement of corn with PM significantly improved ($P < 0.05$) growth and feed conversion. However, there were no additional benefits due to enzyme supplementation. Feeding broilers PM diets did not have any detrimental effects on digesta viscosity, villus height, villus width and villus surface area of the jejunum. On the contrary, in experiment 2, villi were longer ($P < 0.05$) in PM-fed birds than those fed corn diets with or without enzymes. Intestinal loads of *E. coli* were not altered by any of the dietary treatments. But, in experiment 2, both PM diets, with or without enzymes, significantly increased ($P < 0.05$) *Lactobacilli* loads. In both studies, DM, CP and amino acids digestibility were similar between corn and PM diets. However, enzyme supplementation to corn or PM diets increased ($P < 0.05$) DM and CP digestibility. Amino acid digestibility was increased ($P < 0.05$) only in birds fed the corn diet containing enzymes in comparison with those fed the corn diet without enzymes. In conclusion, total replacement of corn with PM in broiler diets caused significant improvements in growth parameters, and *Lactobacilli* populations and villus development of the intestines. Additionally, enzyme supplementation to PM diets increased intestinal DM and CP digestibility.

RESUME

M.Sc. Zootechnie

Millet perlé Canadien: un grain alternatif au maïs dans l'alimentation des poulets à chair

Deux études ont été entreprises pour étudier les effets de replacer le maïs par le millet perlé (MP), soit partiellement ou totalement, dans des régimes du poulet de chair, sans ou en combinaison avec des enzymes exogènes sur la croissance, la digestibilité de la matière sèche, protéines brut (CP) et acides aminé dans l'iléon, la viscosité intestinale, et le développement morphologique et les populations bactériennes dans l'intestin. Dans l'expérience 1, les traitements diététiques étaient: un régime standard de maïs-soja, et la même diète dans laquelle le maïs a été remplacé par le MP à 25, 50, 75 ou 100%. Dans l'expérience 2, les traitements diététiques comprenait: 1) un régime de maïs-soja; 2) un régime de MP-soja; 3) le régime 1+enzymes; et 4) le régime 2+enzymes. Tous les régimes avaient la même teneur en azotes et calories. Les régimes de MP contenaient moins de soja parce que les graines de MP étaient plus riches en CP et acides aminés que le maïs. En remplaçant le maïs totalement par le MP a permis d'améliorée ($P < 0.05$) la croissance et indice de conversion alimentaire. Toutefois, l'utilisation des enzymes n'avait aucun bénéfice. Les régimes de MP n'avaient aucun effet néfaste sur la viscosité intestinale et la longueur, largeur ou superficie du villus dans le jéjunum. Au contraire, dans l'étude 2, les villus étaient plus longs ($P < 0.05$) chez les poulets consommant les régimes du MP que celles nourrit avec les diètes de maïs contenant des enzymes ou pas. Les concentrations d'*E.coli* étaient semblables parmi tous les traitements diététiques. Par contre, dans l'expérience 2, les deux régimes de MP, avec ou sans enzymes, ont augmenté ($P < 0.05$) les concentrations de lactobacilles. Dans les deux études, la digestibilité de la matière sèche, CP et acides aminés étaient semblables entre les régimes de maïs et MP. Cependant, l'ajout des enzymes dans les régimes de maïs et MP avait améliorée ($P < 0.05$) la digestibilité de la matière sèche et de CP. La digestibilité d'acides aminés été élevée ($P < 0.05$) seulement entre les oiseaux alimentés des régimes de maïs contenant des enzymes ou pas. En conclusion, un remplacement total du maïs par le MP dans la moulée du poulet de chair a causé une amélioration dans les paramètres de croissance, et les populations de

lactobacilles et le développement de villus dans l'intestin. En plus, l'ajout des enzymes dans les régimes de MP a permis d'augmenter la digestibilité intestinale de la matière sèche et de CP.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table No. **Page**

LIST OF FIGURES

Figure No. **Page**

LIST OF ABBREVIATIONS

Cal ……………………………………………….	Calorie
ME ……………………………………………...	Metabolizable energy
CP ……………………………………………..	Crude protein
DM …………………………………………….	Dry matter
OM …………………………………………….	Organic matter
NSP …………………………………………….	Non-starch polysaccharides
PM …………………………………………….	Pearl millet

CHAPTER 1: GENERAL INTRODUCTION

1.0 INTRODUCTION

In Canada, chicken production has increased from 615,939 thousands to 640,342 thousands in the year 2003 and 2007, respectively (Statistics Canada, 2009). However, increased chicken production was accompanied by increases in total production costs from $ 1,526484 in 2003 to $ 1,748,663 in 2007 (Statistics Canada, 2009). It is well recognized that 65% of the total poultry production costs are directly associated with costs of feeds.

For decades, corn has been the major utilized grain in poultry diets with inclusion rates often exceeding 50%. Since 2006, the new era of the corn-ethanol industry for biofuel production has increased divergence of corn to this energy sector. Consequently, together with increased consumptions of corn by the growing human population, corn prices have considerably increased over the past years. This unprecedented diversion of corn to the corn-ethanol industry and increasing human consumption have contributed to higher feed costs in poultry production. Therefore, to help alleviate increased production costs and sustain economic feasibility of poultry production, the Canadian poultry industry requires alternative grains to corn.

Wheat, barley and rye have considerably been researched as replacement grains for corn in broiler diets. However, these attempts were unsuccessful as demonstrated by depressed growth and feed efficiency (Almirall et al., 1995; Crouch et al., 1997; Lazaro et al., 2003; Garcia et al., 2008). A reduction in production parameters occurred because of the grains' high contents of anti-nutritional factors, commonly referred as non-starch polysaccharides (NSP). Ingestion of NSP-rich diets affects efficient dietary nutrient digestion and utilization. In broilers, the higher NSP contents in wheat, barley and rye diets in comparison to corn-based diets, detrimentally reduced digestibility of crude protein (Almirall et al., 1995; Nyannor et al., 2007) and metabolizable energy (Choct et al., 1995, Nyannor et al., 2007), and also adversely affected villus development of the intestines (Malouthi et al., 2002; Van Leeuwen et al., 2004). Intestinal villi are vital for nutrient absorption.

The Canadian pearl millet (*Pennisetum glaucum*) is a grain that contains comparable low levels of NSP as corn, but also contains higher concentrations of crude protein and amino acids than corn. Unfortunately, pearl millet has not been used as a feed ingredient in livestock nutrition in Canada. In pigs, crude protein and amino acid digestibility was significantly decreased when these were fed diets formulated with the Canadian pearl millet in comparison with corn (Yin et al., 2002). To-date, none have considered evaluating the Canadian pearl millet as a potential alternative grain to corn in broiler nutrition.

Therefore, two studies were designed to determine: 1) whether Canadian pearl millet grains can partially or totally replace corn in broiler diets; 2) whether exogenous enzyme supplementation to pearl millet diet may help to further exploit pearl millet's grain higher nutritive values. Parameters evaluated included:

- growth performance, feed intake and feed efficiency
- crude protein, amino acids, dry matter and organic matter digestibility of the intestines
- development of morphological structures of the intestines as measured by villi height, villi width and villi surface area
- microbial populations of the intestines, especially *Lactobacilli, Bifidobacteria* and *E. coli*
- digesta viscosity of the intestines

CHAPTER 2: LITERATURE REVIEW

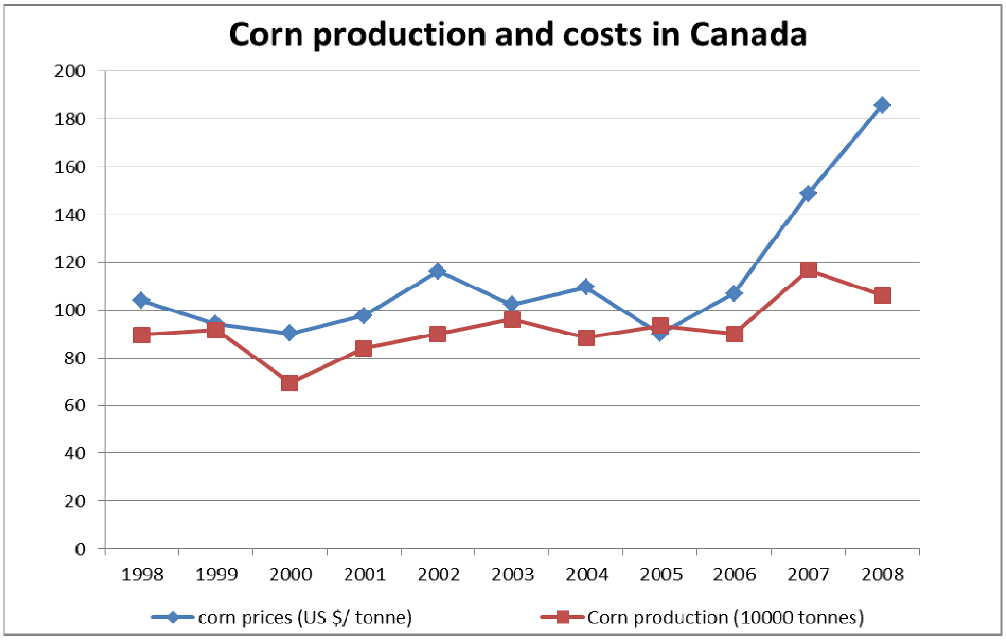
LITERATURE REVIEW

2.1 Corn

Corn, the most utilized cereal grains in formulating broiler diets, is the principal energy source with metabolizable energy as high as 3,350 kcal/kg (NRC, 1994). In broilers, corn contributes to approximately 65% of the total metabolizable energy required for normal growth and development. In general, 2.6 pounds of corn are required to produce 1 pound of chicken meat (Leibtag, 2008). For these reasons, corn is regarded as the golden standard grain in poultry nutrition against which other high energy-containing cereals are compared.

However, over the past years, corn prices have significantly increased. This has been linked with increased corn demands for corn-ethanol production. Therefore, together with increasing corn consumption by the ever-growing human population, corn has become less available as a feed ingredient at affordable prices in the poultry industry. Figure 1.0 shows increased corn prices in Canada over the past decade. Corn prices have known a 74% increase only from the year 2006 to 2008. Therefore, it is highly desirable to identify alternative grains to corn that would alleviate increasing poultry feed costs, sustain economic viability of the industry and favor poultry products to be marketed at affordable prices for poultry consumers.

Figure 1.0. Timely production and costs of corn in Canada



(Source: FOASTAT Database, 2008)

2.2 Wheat, barley and rye as alternative grains to corn in broiler production

2.2.1 Non-starch polysaccharides (NSP): anti-nutritional barriers

Cell walls of cereals are usually composed of complex carbohydrates, commonly termed as non-starch polysaccharides (NSP). But, NSP contents of cereal grains vary according to the genotype or cultivar within and between plant species. External factors such as climatic conditions as well as storage conditions of grains may also contribute to grain's NSP contents (Williams et al, 1997). NSP can be classified into insoluble and soluble types. Insoluble NSP are the cell wall constituents consisting mainly of cellulose, hemicellulose and pectins (Smits and Annison, 1996). Simple laboratory analyses may rapidly provide an indication of the amounts of insoluble NSP in different plant materials. These include (i) Acid Detergent Fibre (ADF) that quantifies cellulose and lignin and (ii) Neutral Detergent Fibre (NDF) which measures cellulose, hemicellulose and lignin.

The soluble type of NSP naturally present in grains has greater relevance in poultry nutrition. It is known that more than 50% of poultry diets comprise grains, and corn in particular. Over decades corn has remained the major utilized grain in poultry diets. This is attributed to its low levels of soluble NSP (0.1 %) in comparison to other cereals such as wheat (2.4%), barley (4.5%) and rye (4.6%) (Choct, 2006). Table 2.1 shows different soluble NSP in corn, wheat, barley and rye. Arabinoxylans are more abundant in rye and wheat, whereas β–glucans are majorly present in barley. Because of the detrimental effects of soluble NSP on chicken growth, these are regarded as anti-nutritional factors. The same effects do not occur with the insoluble type of NSP. Details about the negative effects of soluble NSP on chicken growth are discussed below.

Table 2.1. Types and amounts of soluble NSP (% DM) present in some cereal grains[1]

Cereals	Arabinoxylans	ß-glucans	Galactomannans	Uronic acid	Total
Wheat	1.8	0.4	0.2	-	2.4
Rye	3.4	0.9	0.2	0.1	4.6
Barley	0.8	3.6	0.1	-	4.5
Corn	0.1	-	-	-	0.1

[1]*Adapted from Choct (2006)*

2.2.2 Proposed mechanisms for anti-nutritional effects of soluble NSP

The exact mechanism by which soluble NSP affects growth in chickens is still unknown. But, several mechanisms have been proposed. Soluble NSP have high water binding capacities and therefore interact with water molecules to produce a viscous solution (Smits and Annisson, 1996). Thickening of digesta in the intestine increases digesta retention time, restricts access to endogenous digestive enzymes for digestion, and increases the thickness of unstirred water layer of the intestinal brush border which reduces diffusion and transport of nutrients across the intestinal epithelium. Overall, soluble NSP adversely affects digestive processes and nutrient absorption in the intestines. This is the most accepted and mostly referred mechanism about soluble NSP in poultry.

Soluble NSP such as pectins have a high charge density at a given pH due to the presence of acidic groups (Smits and Annison, 1996). Thus, cations in the form of vital minerals (calcium) form bridges with NSP, thereby trapping nutrients. The gelling properties and viscosity of NSP are also increased when NSP come into contact with other ions in the intestinal tract through hydrophilic or hydrophobic interactions. It is also thought that soluble NSP interact with the intestinal wall in a way to modify the action of peptide hormones and affect secretions of endogenous proteins (Angkanaporn, 1994). Furthermore, increases in viscosity may slow down peristaltic movement causing endogenous secretions of proteins and amino acids as a feedback mechanism (Angkanaporn, 1994).

The effects of soluble NSP in reducing nutrient digestion and absorption in the intestines causes detrimental effects on growth, and development of morphological structures and bacterial populations of the intestines.

2.2.3 Effects of wheat, barley and rye diets on nutrient digestibility, growth and feed conversion

Marquardt et al. (1994) observed a significant decrease in bodyweight when broilers were fed diets formulated with wheat (103 g), barley (99 g) or rye (88 g) instead of corn (123 g) at 14 d of bird age. Similarly, at 21 d of age, feeding broilers a barley-based diet significantly reduced bodyweight gain (24.2 vs 30 g/d) and feed intake (38.4 vs 43.6 g/d) when compared to birds fed a corn-based diet (Almirall et al., 1995). Weight gain (178 vs 313 g), feed intake (397 vs 478 g) and feed efficiency (0.44 vs 0.68) were also reduced when broilers were fed a rye diet than a corn-based diet at 18 d of age (Mathlouthi et al., 2002). Lazaro et al. (2003) also observed depressed bodyweight gain (31.7 vs 40.1 g/d) and feed conversion ratio (1.96 vs 1.66) at 25 d of age when broilers were fed rye than corn diets. Feed conversion ratio was also inferior (1.49 vs 1.25) when birds were fed wheat than corn–based diets at d 21 (Crouch et al., 1997). In all these studies, depressed performance due to wheat, barley and rye diets was attributed to higher soluble NSP contents of the diets that significantly increased digesta viscosity. A study with laying hens showed a significant increase in digesta viscosity coefficients when birds were fed wheat (11.1 cP), barley (33.2 cP) and rye (111.9 cP) diets in comparison to a control maize diet (3.9 cP) (Lazaro et al., 2003).

An increase in digesta viscosity is negatively correlated with intestinal digestibility of nutrients. Metabolizable energy was consistently reduced when broilers were fed diets containing wheat (14.52 vs 16.65 MJ/Kg of DM) or a wheat-barley (12.91 vs 13.68 MJ/kg of DM) diet in comparison with birds fed a standard corn diet (Choct et al, 1995; Mathlouthi et al., 2002). Similarly, Marquart et al. (1994) observed a significant decrease in apparent crude protein digestibility when rye (81.6 vs 89.7 %), wheat (87.7 vs

89.7 %) or barley (84.6 vs 89.7 %) diets were fed to broilers rather than a corn-based diet. Angkanaporn et al. (1994) demonstrated that addition of wheat pentosans to broiler diets markedly reduced the apparent ileal digestibility of amino acids and significantly increased losses of endogenous amino acids (Table 2.1). Obviously, these negative effects caused broilers to have reduced bodyweight. When arabinoxylans were added to broiler diets, Williams et al. (1997) reported increases in losses of endogenous amino acids at low levels of arabinoxylans (15 g/kg), but protein breakdown and impaired amino acid absorption occurred at higher arabinoxylan concentrations (35 g/kg). Inferior fat digestibility (80 vs 83.16%) and starch digestibility (88.37 vs 94.74%) were also recorded when birds were fed a rye-based diet as compared to a corn based diet (Viveros et al., 1994). When comparing a corn-based diet with a rye-based diet, total bile acid concentrations were significantly lowered in the rye-based diet (7.08 vs 13.56 mg/g) leading to reduced crude fat digestibility (25.2 vs 62.8 %) (Mathlouthi et al., 2002).

Table 2.2. Effects of wheat pentosans on ileal apparent amino acid digestibility (%) and endogenous amino acid secretions (g kg^{-1} of DM) in 21-d old broilers[1]

	Amino acid digestibility			**Endogenous amino acid secretions**		
Amino acids	Control	15 g kg^{-1}	35 g kg^{-1}	Control	15 g kg^{-1}	35 g kg^{-1}
Lysine	60.3 ± 3.70[a]	29.8 ± 4.13[b]	22.9 ± 6.61[b]	0.99 ± 0.11[a]	2.02 ± 0.18[b]	1.79 ± 0.12[b]
Methionine	95.5 ± 0.65[a]	90.7 ± 1.85[a]	87.1 ± 4.00[b]	0.11 ± 0.02[a]	0.22 ± 0.06[a]	0.21 ± 0.06[a]
Threonine	62.4 ± 3.99[a]	36.1 ± 4.98[b]	48.2 ± 2.72[b]	3.05 ± 0.37[a]	5.26 ± 0.43[b]	3.47 ± 0.11[a]
Means	96.6 ± 0.34[a]	94.6 ± 1.37[a]	88.5 ± 2.91[b]	30.2 ± 2.71[a]	53.9 ± 4.91[b]	40.2 ± 1.96[a]

[1]*Adapted from Angkanaporn et al. (1994)*

2.2.4 Effects of wheat, barley and rye diets on intestinal morphological development

Counting the number of villi on a surface area and measuring villi height are common reliable methods to investigate development of morphological structures in chickens (Jeurisson, 2002). According to Caspary (1992) the surface area for nutrient absorption may be reduced when villi are shorter or when villi are more tongue-shaped instead of finger-shaped. Villi that are zigzagged/ridged/fingered shape have larger surface area that allows greater absorption of nutrients. Generally, shorter villi are indicative of fewer absorptive and more secretory cells that cause less absorption of nutrients, thereby impairing growth.

Diet type or its contents of soluble NSP has a major role on intestinal morphology, in particular villi shapes and surface area. For instance, addition of high-methylated citrus pectin as a soluble NSP to a corn diet significantly reduced the number of zigzag patterns and ridge-shaped villi when compared to a control corn diet or one containing low levels of methylated citrus pectin (Langhout et al., 1998). In fact, villi were mostly tongue-shaped and leaf-shaped. In another trial with broilers, Leeuwen et al. (2004) also observed reduced zigzag patterns in villi of birds fed diet supplemented with pectin. Replacement of corn with barley in broiler diets caused shortening, thickening and atrophy of villi (Viveros et al., 1994). Similarly, broilers fed rye-based diets had shorter villi (579 vs 686 μm) and reduced surface area (0.16 vs 0.27 μm^2) when compared to others fed a corn-based diet (Mathlouthi et al., 2002). Yasar et al. (1999) also observed increased digesta viscosity and reduced villi height when feeding broilers wheat-based diets. All these findings about increased digesta viscosity due to wheat, barley and rye diets correlated positively with reduced nutrient digestibility and poor growth.

2.2.5 Effects of wheat, barley and rye diets on intestinal bacterial populations

Like human and other animal species, the chicken gastrointestinal tract harbors a community of different types of bacteria, both beneficial and pathogenic. These bacteria significantly influence the uptake and utilization of nutrients (Steenfeldt et al., 1995; Choct et al., 1996; Smits et al., 1997) or interact with the physiological and immunological status (Klasing et al., 1999) of the birds. It is well-known that compositions of the bacterial flora, with regards to proportions of pathogenic and beneficial bacteria, are highly variable and are greatly influenced by diet types and dietary additives including prebiotics, probiotics and exogenous enzymes.

Pathogenic bacteria, including *Salmonella*, *E. coli* and *Clostridium perfringens*, produce toxins that may cause fever, diarrhea and necrosis in chickens (Weltzien, 2003; Gabriel et al., 2006). Other harmful effects of pathogenic bacteria to the hosts include damage to intestinal microvilli and reduced nutrient absorption. *Clostridium perfringens*, which cause necrotic enteritis, is reputed to cause serious economic losses in commercial chicken production. On the other hand, as beneficial bacteria, *Lactobacilli* have been shown to suppress proliferation of pathogenic bacteria (Reid et al., 1988; Spencer and Chesson, 1994; Pascual et al., 1999; Fuller, 2001). Therefore, it is highly desirable that the intestinal microflora are rich in beneficial bacterial strains such as *Lactobacilli* and *Bifidobacteria*.

When broilers were fed wheat or wheat/rye based diets rather than a corn-based diet, the populations of pathogenic bacteria, namely *Enterobacteria*, *Clostridia* and *Enterococci* were significantly increased (Hubener et al., 2002; Apajalahti et al., 2004). Annett et al. (2002) demonstrated higher proliferation of *C. perfringens* type A in wheat (5.83×10^8 CFU/ml) and rye (5.9×10^8 CFU/ml) diets when compared to a corn (3.78 x

10^8 CFU/ml) diet. Similarly, Mathlouthi et al. (2002) showed that inclusion of wheat and barley in broiler diets caused significant increase in the intestinal concentrations of *E. coli* and *Lactobacilli* and that overgrowth of these bacterial groups depressed performance. Finally, Riddel and Kong (1991) observed an increase in mortality due to necrotic enteritis when birds were fed diets containing wheat, rye, barley than corn.

2.3 Pearl millet as an alternative grain to corn in broilers

2.3.1 Agronomic characteristics of pearl millet

Pearl millet (*Pennisetum glaucum*) is native to the western edges of the Sahara desert and is commonly grown as a forage and grain crop in arid areas of Africa and India (Hidalgo et al., 2004). With 260,000 km^2 being grown worldwide, pearl millet grain is ranked as the world's fifth most important cereal in order of economic importance (Poncet et al., 2000). Among the different types of millets, pearl millet grain accounts for approximately 50% of the total world production of millets. In the United States, for example, cultivation of pearl millet has reached nearly 1.5 million acres. In addition to being used as forages in livestock production, pearl millet grain is now increasingly being exploited in livestock feed in several countries, excluding Canada. Since 1991, pearl millet grain has been integrated in animal feed formulations in the US. Today, the grain is utilized mostly in poultry diets.

Pearl millet's agronomic characteristics have favored its extensive cultivation in many countries around the world. Pearl millet is early maturing, well-adapted to drought conditions, grows well in sandy and acid soils with low fertility, drought resistant, and requires about 70% less fertilizer for cultivation than corn (AERC, 2002; Farrel et al., 2005). Moreover, the grains of pearl millet contain antifungal cysteine-protease inhibitors

(Joshi et al., 1998) which decrease contamination by aflatoxins and fumonisins during the periods of pre-harvest and storage (Wilson et al., 2006). When ingested, these toxins may cause metabolic disorders and other diseases.

2.3.2 Nutritional values of pearl millet

The nutritive values of pearl millet are greatly influenced by genotype and external factors such as climatic conditions and cultural practices (Burton et al., 1972). Nevertheless, pearl millet contains better nutritive values than corn. Table 2.3 summarizes that gross energy (4257- 4347 kcal/kg), crude protein (10 - 16%) and fat (1.9 - 4.5%) contents were constantly higher in pearl millet of different types than corn. In addition, pearl millet contains a more well-balanced essential amino acids profile than corn. According to the NRC, pearl millet contains higher methionine (0.25 vs 0.18%), lysine (0.45 vs 0.27%), threonine (0.48 vs 0.32%), cysteine (0.24 vs 0.19%) and arginine (0.74 vs 0.44%) levels than corn (NRC, 1994). In comparison to corn, pearl millet is also richer in omega-3 fatty acids concentrations, including stearic (3.9 vs 2.4%), linolenic (3.7 vs 1.2%), palmitic (20.1 vs 14%) (Burton et al., 1972). Evidently, in terms of nutritional values, these data demonstrate the superiority of pearl millet grains than corn.

Table 2.3. Nutritional values of pearl millet grains and corn

Nutrient composition (%)	Pearl millet	Corn	References
	4,347	4,154	Lawrence et al. (1995)
Gross energy, kcal/kg	4,132	3,788	Adeola and Orban (1995)
	4,257	3,951	Ragland et al. (1997)
	16.0	8.0	Burton et al. (1972)
	11.1	7.5	Adeola and Orban (1995)
Crude protein	13.1	8.1	Ragland et al. (1997)
	12.0	8.1	Lawrence et al. (1995)
	14. 3	10.7	Hill and Hanna (1990)
	4.5	3.9	Burton et al. (1972)
	5.06	1.51	Adeola and Orban (1995)
Fat	6.7	2.9	Lawrence et al. (1995)
	1.9	2.3	Gelaye et al. (1997)

2.3.3 Applications of pearl millet in poultry nutrition

2.3.3.1 Broilers

Over decades, corn has remained the most utilized grain in formulating broiler diets. But, during the past few years, pearl millet has received much attention in formulating poultry diets, mostly in the US, due to its higher nutritional values. On a few studies have been published on the effects of feeding broilers a pearl millet- than corn-based diets on growth performance.

Total replacement of corn by pearl millet in broiler diets showed similar effects on bodyweight (2218 vs 2142 g), feed intake (3949 vs 4091 g) and feed conversion (2.24 vs 2.45) (Clement et al., 2010). When 50% corn was substituted with pearl millet, Davis et al. (2003) observed that broilers had comparable growth and carcass yields. Bodyweight gain was also similar when broilers were fed a standard corn diet or one in which 20% corn was replaced with pearl millet (Hidalgo et al., 2004). In another study, replacing 75% corn with pearl millet had no negative effects on bodyweight and feed conversion (Manwar et al., 2008). As explained by the authors, similar growth was achieved due to lower gross energy metabolizability (69.56 vs 72.67 %) but similar dry matter metabolizability in birds fed diets containing 75% pearl millet than corn.

However, Rag et al. (2003) observed significantly improvements in growth and feed efficiency when corn was substituted with 25% or 75% pearl millet in broiler diets. Indeed, in this study, the pearl millet diet was more efficiently converted into meat or growth. Similarly, significant improvements in bodyweight (1632 vs 1550 g), with unchanged feed conversion (2.31 v/s 2.20), were observed when broilers were fed diets that contained 25% pearl millet than corn diet (Tornekar et al., 2009).

2.3.3.2 Layers

When 26 weeks-old layers were fed diets formulated with corn or pearl millet of different varieties (Baioda, Kordofani, Dimbi), no detrimental effects were observed on egg production and egg weight (Salah et al., 1998). In fact, egg shell thickness was improved when hens were fed pearl millet-based diets; this effect was associated with pearl millet's higher mineral contents. Results of this study are summarized in Table 2.4. Similarly, inclusion of 5 – 40% pearl millet than corn in layer diets did not adversely affect egg production or starch digestibility (Garcia and Dale, 2006). On the other hand, Filardi et al. (2005) observed a reduction in egg production (79.72 vs 87.56 %) and egg weight (57.37 vs 59.65 g) when layers were fed pearl millet-based diets. Additionally, a total replacement of corn with pearl millet in layer diets (44 to 56 weeks) caused a significant decrease in egg production (66.7 vs 74.1 %) and egg weight (56.6 vs 58.8 g) (Mehri et al., 2010). But, both egg production and egg weight were not affected when 75% corn was replaced with pearl millet.

Table 2.4. Effects of corn and pearl millet diets on egg production parameters in layer chickens

Parameters	Corn	Baioda	Kordofani	Dimbi
Egg production (%/d)	83	79	85	78
Mean egg weight (g)	79	80	81	79
Shell thickness (mm)	0.37	0.40	0.43	0.43

Considering pearl millet's high concentrations of stearic (3.9%), linolenic (3.7%) and palmitic (20.1%) acids (section 2.2), attempts have been made to produce omega-3 rich eggs (n-3 linolenic fatty acids) upon feeding layers with pearl millet diets. Reports indicate that n-3 fatty acids can help prevent coronary heart disease, hypertension and type 2 diabetes. When feeding layers pearl millet diets, Amini et al. (2007) reported that eggs contained higher levels of n-3 linolenic (1.3 vs 1.0 mg/g of yolk) and mo-unsaturated (170 v/s 156 mg/g of yolk) fatty acids than when fed corn diets.

2.3.4 Canadian pearl millet: a hybrid variety

Despite pearl millet's higher nutritive values than corn, the grain has no application in the Canadian poultry industry, unlike the US. A major constraint lies in unavailability of a nutritionally-rich pearl millet variety that could adapt well to the Canadian harsh growing conditions and relatively short summer season. In Canada, pearl millet is mostly being utilized for crop rotation purposes to suppress root lesions nematodes.

A new hybrid Canadian pearl millet variety (CGPMH-90) has recently been developed through extensive breeding programs by Agriculture Environmental Renewal Canada Inc. (AERC Inc., Ontario, Canada). This variety is early maturing (100 days) and yields 3 to 3.5 tons grains per hectare under non-irrigated and drought conditions. Evidently, there is now increasing interests in cultivating the hybrid pearl millet variety and exploring its potential application as a feed ingredient in the Canadian poultry industry.

2.3.4.1 Nutritional values of the Canadian pearl millet

The nutrient compositions of the Canadian pearl millet in comparison to corn are presented in Table 2.5. The Canadian pearl millet grains are richer in crude protein, crude fat and amino acids. Additionally, pearl millet contains higher concentrations of the most limiting amino acids in broiler nutrition, including lysine, methionine and threonine. Crude protein and essential amino acids are critical nutrients in diet formulation for protein synthesis and meat deposition in the fast growing meat-type broiler chickens. Deficiency or inadequate levels of these nutrients are known to reduce meat yields and increase carcass fat deposition (Corzo et al., 2008), that lead to productivity and economic losses. Whereas deficiency of the above-mentioned amino acids in corn requires incorporation of relatively large amounts of soybean meal and frequently amino acid supplementation in the synthetic form during diet formulations, pearl millet's higher crude protein and amino acids contents may help reduce or alleviate inclusion rates of these dietary ingredients.

In broilers, different organs and muscles show different rates of protein synthesis. Although nutritive values of the Canadian pearl millet has been researched and determined, digestibility of these nutrients in broilers is not yet reported. So far, only one study has evaluated nutrient digestibility of the Canadian pearl millet in monogastric animals. This study was conducted with growing pigs. Contrary to what was expected, feeding pigs the Canadian pearl millet than corn significantly reduced intestinal digestibility of crude protein (79.6 vs 89.9 %) (Yin et al., 2002). Additionally, pearl millet diet reduced the standardized ileal digestibility of amino acids, including the most limiting ones (lysine, methionine and threonine). Digestibility values were lysine (79.5 vs 85.9%), methionine (77.4 vs 89.8%) and threonine (83.3 vs 96.4%). Depressed crude protein and amino acid digestibility was attributed to higher contents of non-starch polysaccharides, namely ADF (82.2 vs 78.0 g/kg dry matter) and NDF (259.1 vs 207.3 g/kg dry matter), in pearl millet than corn diets. Unfortunately, bodyweight was not measured in the study.

Table 2.5. Nutritional values of Canadian pearl millet and corn

Nutrients	Canadian pearl millet	Corn
Crude protein	127.6	87.8
Crude fat	66.4	31.2
Starch	535	588
Amino Acids		
Arginine	6.8	4.5
Cysteine	2.6	2.0
Histidine	3.2	2.6
Methionine	2.8	1.7
Leucine	13.7	12.2
Lysine	4.5	3.2
Isoleucine	5.5	3.3
Phenylalanine	6.8	5.0
Threonine	5.3	3.8
Tyrosine	3.4	2.5
Valine	7.7	4.8

[1]Adapted from Yin et al. (2002)

2.4 Exogenous enzymes

Emergence of the modern enzyme technology was born in 1874 when the enzyme 'rennet' was extracted from the calves' stomach and refined (Bedford and Partidge, 2000). Since then, identification, extraction and production of enzymes on a commercial scale have rapidly progressed. By definition, an enzyme is a protein or protein-based molecule that acts as a catalyst to speed up chemical reactions involving the conversion of substrates into specific products. Nowadays, enzymes are extensively used in detergent, paper, leather, textile, and food and beverage industries (Bedford and Partidge, 2000). Indeed, exogenous enzymes are also increasingly being used in the livestock industry with objective to improve efficiency of feed utilization leading to increased productivity.

2.4.1 Applications of exogenous enzymes in the poultry industry

In livestock production, the poultry industry is the largest user of exogenous enzymes which are regarded as an important ingredient in diet formulations (Rowe et al., 1999). There are considerable evidences showing that chickens, as monogastric livestock species, have limited capacities to produce sufficient amounts of endogenous enzymes for the efficient digestion of large quantity of different feed types (Sell et al., 1989; Nitsan et al., 1991; Nir et al., 1993). This justifies the need to offer chickens with highly digestible diets to maximize feed digestion and hence productivity in commercial production. Starting from the time of hatch, the fast growing broiler chick consumes an increasing amount of feed that is highly desirable for maximum meat deposition. However, Noy and Sklan (1997) reported that lipase secretion was constant per g of feed consumed between the age of 4 to 21 d. Additionally, bile secretions increased up to 21 d of bird age but then decreased (Krodgahl et al., 1985). In the pancreas, maximum amylase and lipase activities occurred on 8 d of age whereas trypsin and chymotrypsin reached optimum activities on d 11 (Nitsan et al., 1991). The later finding suggests that activities of different endogenous enzymes may not coordinately be regulated for optimum efficiency of feed digestion and utilization in chickens. Evidently, depending on the type of feed consumed, chicken may encounter digestibility difficulties, reduced growth and compromised health.

2.4.2 Exogenous enzymes: Modes of actions and benefits to the poultry industry

As discussed above, wheat, barley and rye contain significant amounts of different types of NSP. On the other hand, chickens have limited capacities in secreting NSP degrading enzymes. For instance, broilers fail to cleave the β-1,4 and β -1,3 glycosidic bonds in β-glycans and β-1,4 glycosidic bonds in cellulose (Smits and Annison, 1996); these are NSP commonly present in all cereals. Hemicellulose, which contains variable levels of arabinoxylans, arabinoglucans, galactomannans and xyloglucans, is also not digested by chickens (Smits and Annison, 1996). Failure to adequately digest in-feed NSP compromises feed digestibility and growth in broiler chickens.

Fortunately, exogenous enzymes such as cellulases, xylanases and β-glucanases have the ability to degrade NSP (Williams et al., 1997; Bedford and Schulze, 1998) causing the release of trapped nutrients for utilization by host birds. The ability of exogenous enzymes to digest various types of NSP has been demonstrated in numerous *in vitro* and *in vivo* studies. A few examples are presented in Table 2.6.

Table 2.6. Effects of enzymes on non-starch polysaccharide (NSP) concentrations (g/kg)

Feed ingredients	NSP	Without Enzymes	With Enzymes	References
Wheat (*in vitro*)	Arabinose	19.9	14.8	Meng et al. (2005)
	Xylose	24.7	15.8	
Soybean meal (*in vitro*)	Arabinose	20.2	12.0	
	Xylose	25.5	10.4	
Soybean meal (*in vivo*)	Insoluble NSP	3980	3369	Kocher et al.(2002)
	Soluble NSP	298	221	

To, therefore, complement the insufficiency of endogenous enzymes secreted by chickens, exogenous enzymes are routinely incorporated into broiler diets during diet formulation. This practice important to sustain high enzymatic activities in fast-growing broilers is critical for efficient feed digestion and growth maximization. For example, when the enzyme β-glucanase was added into barley-based diets, activities of amylase (24.4 vs 15.7 U x 10^{-4} g) and lipase (31.6 vs 24.9 U x 10^{-3} g) were considerably increased that correlated well with increased digestion and utilization of feed nutrients and improved growth (Almirall et al., 1995). Addition of exogenous enzymes in corn-based diets also increased enzymatic activities, including those of sucrases (29.8 vs 21.8 U/mg of protein) and maltases (41.0 vs 21.5 U/mg of protein) when compared to un-supplemented diets (Pinheiro et al., 2004). Table 2.7 shows more details about the modes of action and expected benefits of commonly used exogenous enzymes in broiler nutrition.

Table 2.7. Modes of action and benefits of common exogenous enzymes in broilers

Enzymes	Modes of action	Expected benefits
β-Glucanases	Convert β-Glucans into oligosaccharides + glucose	Reduce sticky droppings and improve feed utilization
Cellulases	Convert cellulose into glucose + other saccharides	Improve energy availability
Xylanases	Arabinoxylans to arabinose and other products	Improve litter quality and improve feed utilization
Phytases	Increase availability of phosphorus	Reduce supplementation of inorganic phosphorous
Proteasease	Convert protein into amino acids + peptides	Increase growth rate

2.4.3 Effects of exogenous enzymes on digesta viscosity of the chicken intestines

As presented in section 2.2.2, soluble NSP such as arabinoxylans and β-glucans create conditions of viscous digesta that detrimentally affect feed digestion and absorption of digested nutrients. However, several studies have shown that addition of exogenous enzymes into broiler diets can help alleviate the deleterious effects of increased viscosity caused by soluble NSP of the diets (Table 2.8).

Table 2.8. Effects of dietary enzymes on intestinal digesta viscosity in broilers

Diets	Digesta viscosity (mPa.s)		References
	Without Enzymes	**With Enzymes**	
Barley	13	2	Almirall et al. (1995)
	29	3	
	2.58	1.72	Shirzadi et al. (2009)
	311	8	Garcia et al. (2008)
Wheat	20.28	10.36	Choct et al. (1995)
	3.6	2.6	Jia et al. (2009)
	3.36	1.79	Basmacioğlu Malayoğlu et al. (2010)
	7.32	4.15	Shakouri et al. (2009)
Rye	140	34	Jozefiak et al. (2007)
Barley/Wheat	2.51	1.43	Shirzadi et al. (2010)
Corn/Wheat	2.77	2.17	Lu et al. (2009)
Corn (*in vitro*)	0.37	0.02	Mathlouthi et al. (2003)

2.4.4 Effects of exogenous enzymes on growth performance in broilers

It is now clear that rye, wheat and barley grains which contain high levels of soluble NSP as anti-nutritive factors cannot be incorporated into broiler diets unless exogenous enzymes are adequately utilized. Evidently, enzymes addition to wheat, barley and rye diets have been shown to significantly improve growth and feed conversion in broilers (Table 2.9). Although corn is considered to be highly digestible, it also contains anti-nutritive factors such as enzyme inhibitors (Cowieson et al., 2005). This observation has been evidenced by significant improvements in growth and feed conversion when broilers were fed corn-based diets supplemented with enzymes (Gracia et al., 2003; Khan et al., 2006; Abudabos et al., 2010).

Table 2.9. Effects of dietary enzymes on growth and feed conversion in broilers

Diets	Bird age (d)	Bodyweight (g) / Bodyweight gain (g/d)		Feed conversion		References
		Without enzymes	With enzymes	Without enzymes	With enzymes	
Wheat	1 -22	99	109	2.15	2.07	Marquardt et al. (1994)
	1 – 27	306	397	0.40	0.51	Choct et al. (1995)
Rye	1-22	85	105	2.06	1.93	Marquardt et al. (1994)
	4 – 25	63.6	66.7	1.96	1.71	Lazaro et al. (2003)
Corn/Rye	28	877.6[a]	1,014[a]	1.74	1.57	Cowieson and Adeola (2005)
Wheat / Barley	4 – 20	438[1]	619	1.645	1. 05	Mathlouthi et al. (2002a)
Barley	1 -21	41.4	49.8	1.85	1.62	Garcia et al. (2008)
	1-22	103	119	2.43	2.07	Marquardt et al. (1994)
	1 – 28	873[a]	1,089[a]	1.90	1.58	Viveros et al. (1994)
Corn	0 – 42	55.1	57.7	1.59	1.57	Gracia et al. (2003)
	0 – 42	1,450[a]	1,876[a]	2.27	1.99	Khan et al. (2006)
	1 – 42	2,462[a]	2,562[a]	1.94	1.83	Abudabos et al. (2010)

[a]Values represent bodyweight (g)

2.4.5 Effects of exogenous enzymes on nutrient digestibility

Addition of enzymes to broiler diets has also been shown to increase digestibility of feed nutrients. Marquardt et al. (1994) observed significant increases in dry matter digestibility in broilers fed barley (73.5 vs 67.5%) and rye (70.2 vs 64.6%) diets supplemented with enzymes in comparison to the same diets without enzymes. In another study, Almirall et al. (1995) showed that enzyme addition increased dry matter contents (18 vs 16.5 g/100 g of digesta) in the small intestines of broilers when fed barley-based diets. Enzyme supplementation also increased dry matter digestibility when broilers were fed corn-based diets (Gracia et al., 2003; Khan et al., 2006).

Similarly, digestibility of metabolizable energy was increased by 12%, 10% and 4% in barley, rye and wheat respectively when diets were supplemented with enzymes (Marquardt et al., 1994). Several other studies have also shown increases in digestibility of apparent metabolizable energy when enzymes were added to diets formulated with different grain cereals (Choct et al., 1995; Mathlouthi et al., 2002a; Mathlouthi et al., 2002b; Meng et al., 2005). Apparent crude protein digestibility improved from 60.89% to 63.82% with enzyme supplementation to wheat-based diets (Wang et al., 2005). Improvements in amino acid digestibility due to dietary enzymes have also been demonstrated (Namkung and Leeson, 1999; Ravindran et al., 1999). Table 2.10 shows that enzymes supplementation to barley, rye and corn diets increased digestibility of crude protein and crude fat in broilers.

Table 2.10. Effects of dietary enzymes on crude protein and fat digestibility in broilers

Diets	Crude protein digestibility coefficient		Crude Fat digestibility coefficient		References
	Without Enzymes	With Enzymes	Without Enzymes	With Enzymes	
Rye	0.817	0.867	-	-	Marquardt et al. (1994)
	0.551	0.692	0.367	0.773	Silva et al. (2002)
	0.762	0.853	0.251	0.443	Mathlouthi et al. (2002b)
Barley	0.846	0.897	-	-	Marquardt et al. (1994)
	0.74	0.78	-	-	Onderci et al. (2008)
	0.741	0.834	0.767	0.808	Almirall et al. (1995)
	0.63	0.76	0.38	0.59	Svihus et al. (1997)
Corn	0.753	0.793	-	-	Abudabos et al. (2010)
	0.615	0.681	0.783	0.858	Khan et al. (2006)
	0.791	0.847	-	-	Rutherfurd et al. (2007)

Soybean meal is reputed as the major source of crude protein (45%) and amino acids in broiler nutrition (Lemme et al., 2004). But, digestibility and utilization efficiencies of soybean meal's crude protein and amino acids are compromised by its high NSP (19.2%) levels (Choct, 1997). Enzymes significantly increased ileal digestibility of soybean meal in broilers (Café et al., 2002) and increased protein hydrolysis coefficients (0.24 vs 0.17) *in vitro* (Yu et al., 2006). Odetallah et al. (2005) showed that feeding broilers corn-soybean diets containing lower amino acids amounts and supplemented with enzymes had similar bodyweight (2.02 kg vs 1.97 kg) and feed conversion (1.59 vs 1.59) when compared to corn-soybean diets containing higher amino acids amount and un-supplemented with enzymes.

2.4.6 Effects of exogenous enzymes on intestinal morphological development

Exogenous enzymes also play important roles in improving the development and functioning of morphological structures of the intestines. For instance, Tarachi and Yamauchi (2000) observed longer intestinal villi, and increased enteral absorption and digestibility of nutrients in broilers when fed diets supplemented with enzymes. Enzyme addition to a rye based diet increased villi height (770 vs 579 µm) and villi surface area (0.31 vs 0.16 mm^2) when compared to the same diets without enzymes (Mathlouthi et al., 2002b). The authors also reported that villi height was comparable between broilers fed the rye diet containing enzymes and a normal corn based diet without enzymes. Barley-based diets supplemented with enzymes also showed improvement in villi height (1322 vs 1250 µm) and villi surface area (484.8 vs 480.6 x 10^{-3} μm^2) in comparison to un-supplemented diets (Gracia et al., 2003b). Additionally, villi were longer with less atrophy when barley (Viveros et al., 1994) and wheat (Wu et al., 2004) diets were supplemented with enzymes. Villi height was also increased (615 vs 568 µm) when broilers were fed corn-soybean diets supplemented with enzymes when compared with enzyme un-supplemented diets (Jackson et al., 2004). Therefore, it appears that enzyme supplementation relieved the negative effects of wheat, barley and rye diets on villus development in broilers.

2.4.7 Effects of exogenous enzymes on intestinal bacterial populations

It has been suggested that bacterial population of the intestines greatly influences the development of its morphological structures (Samanya and Yamauchi, 2002; Awad et al., 2006). According to Bedford (2000), enzyme supplementation reduces the populations of intestinal bacteria by increasing the digestibility of NSP that reduces nutrient availability for bacterial growth. Enzyme supplementation to wheat diets significantly increased the intestinal population of beneficial *Lactobacilli* bacteria (7.85 vs 7.10 log CFU g^{-1}) in comparison to the same diet without enzymes (Shakouri et al., 2009). The authors also reported positive correlation between enzymes, and *Lactobacilli* concentrations and villi height (1119.0 vs 971.9 µm). In another study with broilers fed with barley diets, intestinal *Bifidobacteria* concentrations (9.69 v/s 3.62 log CFU g^{-1}) were significantly increased due to enzyme supplementation (Jozefiack et al., 2010).

Addition of enzymes to wheat and barley diets also significantly reduced the intestinal concentration of pathogenic *E. coli* bacteria (5.7 vs 6.5 log CFU g^{-1}) in comparison to enzyme un-supplemented diets (Mathlouthi et al., 2002b). In rye diets, enzyme supplementation also lowered enterobacterial populations (4.42 vs 6.35 log CFU g^{-1}) of the intestines when compared to the same diet without enzymes (Danicke et al., 1999). Hubener et al. (2002) also observed a decrease in enterobacteria counts when wheat/ rye base diets were supplemented with enzymes.

Based on the literature, it is clear that pearl millet can potentially replace corn in broiler diets. Thus, we will evaluate the Canadian pearl millet as potential alternative to corn in broiler nutrition, with or without enzyme supplementations, on growth, intestinal morphological development and microbial populations, and intestinal digestibility of vital nutrients.

Preface to Chapter 3

Chapter 3 comprises a manuscript, co-authored by N. Baurhoo, B. Baurhoo, A. F. Mustafa, and X. Zhao, which has been accepted for publication in the journal of *Poultry Science*. All literature cited in this chapter is listed in the 'References' section at the end of the thesis.

Chapter 3 describes Experiment 1 that has been conducted to investigate the possibility of totally or partially replacing corn by Canadian pearl millet grains in broiler diets. Comparisons were made between the effects of corn- and pearl millet-based diets on growth performance, and nutrient digestibility, digesta viscosity, histomorphological parameters and microbial populations of the intestines.

CHAPTER 3

Comparison of corn- and Canadian pearl millet-based diets on performance, digestibility, villus morphology, and digestive microbial populations in broiler chickens[1]

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Comparison of corn- and Canadian pearl millet-based diets on performance, digestibility, villus morphology, and digestive microbial populations in broiler chickens[1]

3.1 ABSTRACT

A study was undertaken to examine the effects of partially or totally replacing corn with pearl millet in broiler diets on growth performance, jejunal digesta viscosity and histomorphological parameters, ileal CP digestibility, and cecal microbial populations. Two hundred 1-d-old male Ross 508 broilers were randomly assigned to 1 of 5 iso-nitrogenous and iso-caloric dietary treatments (8 cage replicates; 5 birds per cage) and grown over a 42-d experimental period. Dietary treatments included: a standard corn-soybean meal diet and one in which corn was replaced by 25, 50, 75 or 100% pearl millet. All diets contained chromic oxide (0.4%) as an indigestible marker. Body weight and feed intake were recorded weekly throughout 42 d. At d 14, 28 and 42, 8 birds per treatment (1 bird per cage) were euthanized for sample collection and analysis. In comparison to corn, pearl millet grains contained higher CP (14.48 v/s 7.35%, on DM basis) but slightly lower ME (3,093 v/s 3,355 kcal/kg, on DM basis). Total replacement of corn by pearl millet significantly ($P < 0.05$) improved BW and feed conversion. Moreover, in comparison to the standard diet, feeding broilers pearl millet-based diets had no detrimental effects on digesta viscosity, villus height, villus width and villus surface area of the jejunum. Ileal CP digestibility and cecal concentrations of *E. coli*, *Lactobacilli* and *Bifidobacteria* were also similar between corn- and pearl millet-fed birds. It was concluded that substituting corn for pearl millet in broiler diets can improve production responses without causing any adverse effects on nutrient digestibility or bird health.

(**Key words**: pearl millet, non-starch polysaccharides, viscosity, digestibility, broilers)

3.2 INTRODUCTION

Feed accounts for 60 – 65% of total expenditure in broiler production. For decades, corn has been the major utilized feed ingredient with inclusion rates above 50% in most instances. Considering its high ME contents (3,350 kcal/kg; NRC, 1994), corn is the principal dietary energy source that contributes to approximately 65% of broiler ME requirements (Coweison, 2005). In recent years, however, corn has increasingly and preferentially been diverted towards human consumption and corn-ethanol industry rather than the livestock sector. The risks that corn will be less available and marketed at uneconomically higher prices have necessitated identification of alternative grains that could totally or partially replace corn in broiler diets, thereby sustaining economic feasibility of the industry.

Wheat, barley and rye have been investigated over the past years as replacement grains for corn in broiler production. Unfortunately, in comparison to corn, β-glucans (barley) and arabinoxylans (wheat and rye) are major anti-nutritive water-soluble non-starch polysaccharides (NSP) that limit inclusion rates of these grains in poultry diets. These NSP detrimentally increased digesta viscosity (Almirall et al., 1995; Crouch et al., 1997; Lazaro et al., 2003), favored the growth of some pathogenic bacterial species including *E. coli* and *Clostridium perfringens* (Riddell and Kong, 1992; Langhout et al., 1999; Annett et al., 2002), and negatively altered villus height, width, surface area and shape (Langhout et al., 1999; Mathlouthi et al., 2002a). There is compelling evidence indicating that broilers fed wheat-, barley- and rye-diets suffer from reduced DM, starch, CP, amino acids, fat and ME digestibility and absorption (Almirall et al., 1995; Choct et al., 1995, Langhout et al., 1999; Mathlouthi et al., 2002a) that resulted in depressed BW gain and feed conversion ratio (Almirall et al., 1995; Crouch et al., 1997; Mathlouthi et al., 2002a; Lazaro et al., 2003).

In contrast, pearl millet (*Pennisetum glaucum*; **PM)** contains fewer anti-nutritional factors than many other grains (Andrews and Kumar, 1992; Choct, 2006) and possesses unique nutritive values that are attractive to poultry nutrition. Arabinose and xylans are the major water-soluble NSP in PM grains (Hadimani et al., 2001). In comparison to corn, PM possesses comparable (Davis et al., 2003) or better (Andrews and Kumar, 1992) ME levels, and higher amino acid concentrations (Adeola and Orban, 1995; Yin et al., 2002). Furthermore, the digestibility of essential amino acids, namely lysine, arginine, threonine, valine and isoleucine, were higher in pigs fed PM- than corn-based diets (Adeola and Orban, 1995). In broilers fed iso-nitrogenous and iso-caloric PM- and corn-based diets, performance and carcass yields were equivalent or better in the PM groups of birds (Davis et al., 2003; Hidalgo et al., 2004; Manwar and Mandal, 2009). Nevertheless, PM has no nutritional applications in the Canadian poultry industry. A major constraint lies in unavailability of a nutritionally-rich PM variety that could adapt well to the Canadian harsh growing conditions and relatively short summer season. It is also worth mentioning that the concentrations of ME, CP, amino acids and anti-nutritional factors greatly vary between different PM varieties (Buerkert et al., 2001; Mustafa et al., 2008).

There is, now, increasing interest in a new hybrid Canadian PM variety (CGPMH-90, Agriculture Environmental Renewal Canada Inc.; AERC Inc., Ontario, Canada) that has recently been developed through extensive breeding programs. In a study with layers, Amini and Ruiz-Feria (2007) demonstrated that the Canadian PM can fully substitute for corn without affecting BW, feed consumption and egg production. Yet, no study has evaluated the possibility of totally or partially replacing corn by the Canadian PM in broiler diets.

Therefore, the objectives of this study were to investigate the effects of feeding broilers a diet containing corn or different inclusion rates of PM on growth, jejunal digesta viscosity and morphology, ileal CP digestibility, and cecal microbial populations.

3.3 MATERIALS AND METHODS

3.3.1 Bird Husbandry

Two hundred 1-d-old male Ross 508 broilers were obtained from a local commercial hatchery (Couvoir Simetin, Mirabel, Quebec, Canada) and grown over a 42-d experimental period. Birds were randomly assigned to 1 of 5 dietary treatments (8 cage replicates; 5 birds per cage). Throughout the study, caged birds were housed in an environmentally controlled room following a standard temperature regimen that gradually decreased from 32 to 24^{o}C by 0.5^{o}C daily, and under a 20L:4D lighting cycle. Procedures for bird management and care were approved by the Animal Care Committee of McGill University. Birds were group weighed by cage and feed intake was determined at weekly intervals.

3.3.2 Experimental Diets

Birds were fed a corn- or PM-soybean meal based diet ad libitum. The 5 experimental diets included a standard corn-soybean meal diet (**CTL**) and a similar diet in which corn was replaced by 25, 50, 75 or 100% of the Canadian PM (**PM 25**, **PM 50**, **PM 75** and **PM 100,** respectively; AERC Inc.). All diets were in mash form and were formulated to be isoenergetic, isonitrogenous and to meet or exceed NRC (1994) nutrient requirements for macro- and micronutrients (Table 3.1). Diet formulations were based on the least-cost feed formulation. Chromic oxide (4 g/kg) was incorporated in all diets as an indigestible marker for determination of ileal CP digestibility. Corn and PM grains were chemically analyzed for ME, DM, CP, crude fat, total sugar and starch (AgriFood laboratories, Ontario, Canada) and used during diet formulation (Table 3.2); amino acid compositions of PM were as described by Yin et al. (2002).

3.3.3. Sample Collection

At 28 and 42 d of age, one bird per treatment cage (n = 8/treatment) was randomly selected, weighed, and euthanized by electrical stunning and bleeding of the carotid artery. On d 14, jejunal, ileal, and cecal digesta, respectively, from 3 birds were pooled per sample (n = 24/treatment) to obtain enough sample for viscosity, CP digestibility and bacterial analyses, respectively. The intestinal tract was immediately excised and the following segments were collected: jejunum (2 cm from end of duodenum to Meckel's diverticulum), ileum (from the Meckel's diverticulum to 40 mm above the ileo-cecal junction) and ceca (left and right).

3.3.4 Gut Digesta Viscosity

The jejunum contents of respective treatment birds were carefully hand-stripped into 15-mL centrifuge tubes and placed on ice. Jejunal digesta viscosity was determined following the procedure of Choct et al. (2004) with few modifications. Briefly, 2 g of fresh digesta were centrifuged (12,000 x *g* for 10 mins at 4ºC) and 1 mL of the supernatant fraction was used in an advanced rheometer (Model AR 2000EX; TA Instruments, DE) at 40ºC fitted with a CP40 cone and shear rate varying from 5 to 500 / s. The samples did not show any shear thinning at these shear rates. All readings were taken at 42 / s and recorded in centipoises (cPs).

3.3.5 Gut Histomorphology

A 1-cm segment of the jejunum (n = 8/treatment) was washed in physiological saline solution and fixed in 10% buffered formalin. Fixed tissue samples were washed 3 times in 0.1 M phosphate buffered saline (pH 7.4) for 15 min each time, dehydrated using increasing ethanol concentrations at 70% (overnight), 80% (1 hr), 90% (1 hr) and 100% (1 hr over 3 consecutive times), and then embedded in paraffin. Five-micrometer cross sections (3 per sample) were sectioned using a microtome, placed on a glass slide and stained with hematoxylin and eosin. Villus height and width were measured using a phase contrast microscope with integrated image analysis NIS-Element BR v. 2.3 software (Nikon DXM 1200c, Nikon Corporation, Tokyo, Japan). Villus height was measured from the tip of the villus to the top of the lamina propria, whereas villus width was measured at the basal and apical ends, excluding the crypt. Villus surface area was calculated using the formula $(2\pi) * (\text{villus width} / 2) * (\text{villus height})$ (Sakamoto et al., 2000). Ten replicate measurements per bird were averaged and used in statistical analysis.

3.3.6 Microbiological Analysis

Fresh cecal contents of the respective treatment birds (n = 8/treatment) were first mechanically homogenized at room temperature using a stomacher (model 400 Lab Blender, Seward Medical, London, UK) and then diluted 10-fold by weight in buffered peptone water (Fisher Scientific, Ottawa, Ontario, Canada). The homogenized samples were then serially diluted in 0.85% sterile saline solution and used for enumeration of

Lactobacilli, *Bifidobacteria* and *E. coli*. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. *Lactobacilli* were assayed using de Man, Rogosa, and Sharpe agar (Fisher Scientific) and incubated at 37°C for 48 h. *Bifidobacteria* concentrations were determined using Wilkins-Chalgren agar (Oxoid, Nepean, Ontario, Canada), glacial acetic acid (1 mL/L), and mupirocin (100 mg/L; Oxoid) and incubated at 37°C for 3 d (Rada et al., 1999). The use of anaeropacks (Oxoid) in anaerobic jars created anaerobic conditions required for *Lactobacilli* and *Bifidobacteria* growth. Enumeration of *E. coli* was performed by using Rapid *E. coli* 2 agar (BioRad Laboratories, Mississauga, Ontario, Canada), modified using *E. coli* supplement (BioRad), and incubated at 37°C for 48 h. Colonies of bacteria were counted after respective incubation periods.

3.3.7 Ileal Crude Protein Digestibility

From each euthanized bird, the entire ileal digesta contents were collected to obtain sufficient working material for measurements of DM contents and apparent ileal digestibility (**AID**) coefficients for CP. Ileal digesta and diet samples from respective treatment groups were dried overnight in a forced-air oven at 70ºC, ground to pass through a 0.5-mm sieve and stored in airtight containers at -20ºC. Dry matter (method 925.09) was determined according to methods of the AOAC (1990). Crude protein (N x 6.25) of all samples was determined using an N Analyzer (model FP 428, Leco Corp., St Joseph, MI). Chromic oxide concentrations were determined following the method of Fenton and Fenton (1979). Briefly, dried ileal (1 g) and diet (2 to 3 g) samples were accurately weighed, ashed (480ºC for 24 h), and were treated twice with a boiling digestion mixture containing 4 mol/L HCl, sodium molybdate dehydrate (20g / L) and perchloric acid

(70%). Chromic oxide from the supernatant was then measured using a spectrophotometer at 440 nm (Spectronic 21D, Milton Roy Co., Rochester, NY). The AID coefficients for CP were calculated using the following equation:

$$AID\ (\%) = 1 - [CP_{ileal} \times Cr_{diet} / CP_{diet} \times Cr_{ileal}]$$

where CP_{ileal} is the concentration of CP in the ileal digesta (%), Cr_{diet} is the concentration of chromium in the diet (%), CP_{diet} is the concentration of CP in the diet (%), and Cr_{ileal} is the concentration of chromium in the ileal digesta (%).

3.3.8 Statistical Analysis

Data were analyzed by a 1-way ANOVA using the GLM procedure of SAS (SAS Institute, 2003) with cages as experimental units for performance parameters and birds as experimental units for histomorphology, microbiology and nutrient digestibility parameters. Differences among treatment means were tested using Scheffe's multiple comparison *t*-test (SAS Institute, 2003) and statistical significance declared at $P < 0.05$. All microbiological concentrations were subjected to base-10 logarithm transformation before analysis.

3.4 RESULTS

3.4.1 Bird Performance

Overall (d1-42), birds fed PM 100 had greater BW, feed intake and FCR than CTL- and PM 25-fed birds (Table 3.3). At 14, 28, and 42 d of age, birds fed the PM 100 diet were consistently heavier than CTL-, PM 25- and PM 75-fed birds. PM 50-fed birds were also heavier at d 14 and 28 than birds fed PM 25. But, increased BW occurred among birds fed PM 50 than those fed the PM 75 diet at d 14 only. Body weight did not differ between birds fed PM 100 and PM 50 at any time points. There was also no difference in BW between CTL-, PM 25-, PM 50-, and PM 75-fed birds at d 42.

At both d 28 and 42, birds consumed more of the CTL and PM 25 diets than the PM 50, PM 75 and PM 100 diets. Moreover, throughout the entire 42 d, FCR was greater among birds fed the CTL and PM 25 diets than those fed the PM 50, PM 75 and PM 100 diets. However, at d 28 and 42, feed intake and FCR did not differ between PM 50, PM 75 and PM 100. But, at d 14, birds consumed less of the PM 75 diet than CTL, PM 50 and PM 100.

3.4.2 Viscosity, Crude Protein Digestibility, and Histomorphological parameters

At 14, 28 and 42 d, none of the dietary treatments significantly altered the viscosity of jejunal digesta (Table 3.4). But, at d 28 and 42, ileal DM contents was higher in birds fed PM 75 and PM 100 than those fed the CTL and PM 25 diets; there was, however, no difference among dietary treatments at d 14. Additionally, CP digestibility did not differ between dietary treatments at all times. There were also no differences in villus height, villus width and villus surface area of the jejunum among all treated birds at d 28 and 42 (Table 3.5).

3.4.3 Enumeration of *Lactobacilli*, *Bifidobacteria* and *E. coli* in the ceca

There were no major dietary effects on the cecal populations of lactobacilli, *Bifidobacteria* and *E. coli* (Table 3.6). At d 28 only, *E. coli* concentrations were higher in birds fed the CTL, PM 25 and PM 50 diets than in PM 75- and PM 100-fed birds. On the other hand, lactobacilli loads were higher in PM 25-fed birds than birds in the CTL and PM 75, and PM 100 groups at d 14 and d 28, respectively; but, at d 42, lactobacilli loads did not differ among dietary treatments. Lactobacilli loads were also higher in PM 50 than PM 100 birds, but occurred at d 28 only. Finally, *Bifidobacteria* populations were similar among all treatment birds at all times.

3.5 DISCUSSION

Our findings clearly indicate that replacing corn with PM in broiler diets did not adversely affect production responses. On the contrary, in comparison to the CTL diet, feeding broilers PM 100 caused significant improvement in growth and feed efficiency. Additionally, feed efficiency was consistently improved when broilers were raised on diets containing PM at inclusion rates greater than 50%. In another study with broilers, substituting 33% corn by PM in the diets also significantly improved BW (Davis et al., 2003). But, equivalent BW gain and feed efficiency responses were observed when replacing 5 to 75% of dietary corn by PM in broilers (Davis et al., 2003; Hildago et al., 2004; Manwar and Mandal, 2009) and pigs (Lawrence et al., 1995). In layers, partial (50%) or total replacement of corn by PM had no effect on BW, feed conversion and egg production (Collins et al. 1997; Amini and Ruiz-Feria, 2008). Therefore, in agreement with previous reports, results of this study demonstrate that PM can partially or totally replace corn in broiler diets without adversely affecting production responses.

We observed that birds consumed less of the PM-based diets. This may possibly be attributed to differences in feed texture. Due to their relatively smaller size, PM grains were less effectively ground during feed manufacture resulting in coarser PM diets whereas the CTL diet was completely in mash form. Interestingly, improvement in growth occurred despite the marked reduction in consumption of diets containing higher PM levels (50 to 100%) than corn. Considering that energy and CP are essential for growth, the PM diets may be thought to contain higher levels of these nutrients. However, all diets were formulated to be iso-nitrogenous and iso-caloric. Given that the Canadian PM grains were richer in CP than corn (Table 3.2), less soy-bean meal was required during formulation of PM-based diets; this may represent an advantage in alleviating feed costs. Interestingly, however, ileal CP digestibility was not different between PM and corn-fed birds.

The exact mechanism underlying PM effects in improving growth is still not clear, but may be attributed to its amino acid contents. In comparison to corn, PM contains a more well-balanced amino acid profile as well as higher amino acid concentrations (Yin et al., 2002). Whereas corn is markedly deficient in several amino acids, including lysine, methionine, threonine, tryptophan, arginine, valine and methionine (Fernandez et al., 1994) in broiler nutrition, the Canadian PM is richer in lysine, methionine, threonine, arginine, cystine, histidine, isoleucine, leucine, phenylalanine, tyrosine and valine (Yin et al., 2002). Likewise, other PM varieties have been reported to contain greater concentrations of these essential amino acids than corn (Adeola and Orban, 1995). These limiting amino acids are required to maximize protein synthesis and meat deposition in broilers. Moreover, increased growth may be associated with higher analyzed fat contents in PM 100 than the CTL diet (7.76 v/s 4.60% DM basis respectively). Increased dietary fat may be associated with greater soybean oil supplementation during formulation of iso-caloric diets (Table 3.1). Most importantly, however, there is strong evidence that increasing the dietary fat contents or soybean oil supplementation resulted in increased amino acid digestibility (Li and Sauer, 1994; Cervantes-Pahm and Stein, 2008). Not surprising, therefore, that amino acid digestibility was consistently increased when growing pigs were fed PM- than corn-based diets (Adeola and Orban, 1995; Yin et al., 2002). Unfortunately, none of the authors measured growth and feed efficiency. Todate, there are no data about amino acid digestiblity in broilers fed PM diets. The hypothesis that PM's higher amino acid contents contributed to improvement in broiler growth will be tested in our next study. But, it is clear that PM contains attractive nutritional factors which may significantly increase broiler growth and productivity.

Another important finding of this study is the similarity in intestinal digesta viscosity between PM and corn diets. This is the first report revealing the effects of PM on digesta viscosity in broilers. It is known that water-soluble NSP possess the ability to bind water of the digesta, thereby increasing digesta viscosity (Choct and Annison, 1992; Smits et al., 1997; Langhout et al., 1999). A viscous digesta reduces nutrient digestion and absorption by reducing the activities of digestive enzymes, and minimizing the diffusion capacity of nutrients across a thicker unstirred water layer of the intestinal brush border (Smits and Annison, 1996). Although all cereals typically contain NSP, considerable variations in the types and quantities of NSP exist between different grains. Corn (0.1%), the major dietary ingredient in broiler nutrition, has successfully been utilized due to its relatively lower amounts of soluble NSP in comparison to other cereals such as wheat

(2.4%), barley (4.5%) and rye (4.6%) (Englyst, 1989; Choct, 2006). On the other hand, soluble NSP concentrations were reported to be comparable between PM (0.2%) and corn (Choct, 2006). Increased digesta viscosity, reduced nutrient (DM, starch, CP and ME) digestibility and absorption, and depressed BW and feed conversion due to β-glucans and arabinoxylans contents in wheat, barley and rye have previously been reported (Choct and Annison, 1992; Almirall et al., 1995; Mathlouthi et al., 2002a). For these reasons, the replacement of corn by wheat, barley or rye in poultry diets has not been successful unless relying on intensive usage of exogenous enzymes. Enzyme supplements are known to eliminate the anti-nutritive barrier of soluble NSP, thereby addressing the viscosity problem and improving growth due to increased efficiency of nutrient utilization (Marquardt et al., 1994; Mathlouthi et al., 2002b; Lazaro et al., 2003; Shirzadi et al., 2010). The similarity in digesta viscosity between PM and corn diets, because PM did not quantitatively contain more NSP than corn, revealed that PM can be incorporated in equal amounts as corn in broiler diets. This is supported by our results about the similarity in ileal CP digestibity, as herein mentioned.

Feeding broilers PM or corn diets did not alter the intestinal populations of *E. coli*, *Lactobacilli* and *Bifidobacteria*. These results are not surprising given the similarity in intestinal digesta viscosity between PM- and corn-fed birds. Previous reports indicate that an increase in digesta viscosity causes more undigested and fermentable nutrients to reach the lower small intestines which consequently favors bacterial outgrowth. Choct et al. (1996) associated an increase in intestinal concentrations of volatile fatty acids, after the addition of wheat pentosans in chick diets, to increased microbial activity. Increasing digesta viscosity significantly increased the intestinal concentrations of *E. coli*, *Clostridium perfringens*, *Enterococci* and *Bacteriodaceae*, whereas *Lactobacilli* and *Bifidobacteria* populations were unaltered (Riddell and Kong, 1992; Langhout et al., 1999; Annett et al., 2002). In addition to their detrimental intestinal health effects, these pathogenic bacteria compete with the host for nutrients. The greater divergence of nutrients towards bacterial growth may also have contributed to depressed growth among broilers fed wheat, barley or rye diets.

Disrupting the normal microflora may adversely affect the morphological structures of the intestines, thereby reducing the efficiency of nutrient digestion and absorption (Stappenbeck et al., 2002). Moreover, feeding broilers NSP-rich diets has been reported to markedly affect villus length (shorter), width (narrower), shape (ridge-shaped) and arrangement (zig-zag pattern), which significantly reduced the absorptive capacity for nutrients (Viveros et al., 1994; Langhout et al., 1999, Mathlouthi et al., 2002a). However, our findings suggest that feeding broilers PM-based diets had no detrimental effects on intestinal morphology. This is in agreement with our results for viscosity and microflora. The lack of difference in villi height between PM- and corn-fed birds may be explained by the similarity in CP digestibility among dietary treatments; it is known that villi height correlates positively with nutrient absorption (Tarachai and Yamauchi, 2000).

In conclusion, replacing corn with PM in broiler diets resulted in significant improvements in growth and feed efficiency. Interestingly, dietary incorporation of PM required less soybean meal supplementation and caused broilers to reach market weight earlier; these may improve the economics of broiler production. Finally, PM and corn had equivalent effects on intestinal digesta viscosity, microbial populations, morphological development and nutrient digestibility. Therefore, our findings clearly demonstrate that the Canadian PM can fully replace corn in broiler diets.

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Table 3.1. Composition (%) and calculated analysis (%) of experimental diets

	Diet[1]				
Item	CTL	PM 25	PM 50	PM 75	PM 100
Ingredient					
Corn	54.31	41.41	28.05	14.50	0.00
Pearl millet	0.00	14.0	28.50	43.22	58.96
Soybean meal, 48 % CP	38.13	36.51	34.83	33.12	31.29
Soybean oil	3.17	3.61	4.07	4.53	5.03
DL-Met	0.19	0.18	0.18	0.17	0.17
Lys-HCl	0.04	0.07	0.10	0.12	0.15
Vitamin-mineral premix[2]	0.68	0.68	0.67	0.68	0.67
Monocalcium phosphate	1.52	1.57	1.63	1.69	1.76
Sodium carbonate	0.10	0.10	0.10	0.10	0.10
Limestone	1.24	1.24	1.23	1.22	1.21
Sodium chloride	0.22	0.23	0.24	0.25	0.26
Chromium oxide	0.40	0.40	0.40	0.40	0.40
Calculated analysis					
ME, kcal/kg	3,050.00	3,050.00	3,050.00	3,050.00	3,050.00
CP	22.50	22.50	22.50	22.50	22.50
Crude Fat	5.44	6.15	6.89	7.64	8.44
Lys	1.35	1.35	1.35	1.35	1.35
Met	0.55	0.55	0.55	0.55	0.55
Ca	0.90	0.09	0.90	0.90	0.90
Available P	0.45	0.45	0.45	0.45	0.45

[1]CTL: corn-based diet; PM 25, PM 50, PM 75 and PM 100: a similar diet in which corn was replaced by 25 %, 50 %, 75 % or 100 % with PM respectively.

[2]Supplied per kilogram of diet: vitamin A, 6,000 IU; vitamin D, 3,000 IU; vitamin E, 50.21 IU; vitamin B12, 0.03 mg; vitamin K, 2.04 mg; biotin, 0.15 mg; choline, 1,300.5 mg; folic acid, 1.47 mg; niacin, 48.99 mg; panthothenic acid, 13.35 mg; riboflavin, 6.4 mg; thiamine, 2.23 mg; pyridoxine, 3.20 mg; Cu, 12.63 mg; Fe, 22.6 mg; Mn, 108.53 mg; Zn, 108.03 mg; Co, 0.46 mg; I, 0.8 mg; and Se, 0.39 mg

Table 3.2. Analyzed chemical composition of corn and pearl millet grains[1]

Item	Corn	Pearl Millet
ME, kcal/kg	3,355	3,093
DM, %	88.26	88.96
CP[2] (N x 6.25)	6.49	12.88
Crude Fat[2]	2.87	5.41
Total sugar[2]	1.77	1.65
Starch[2]	57.43	40.83

[1]Values represent the means of 4 analyses per sample

[2]data presented as %, on an as-is basis

Table 3.3. Effects of dietary treatments on BW, feed intake, and feed conversion of broiler chickens[1]

Age	Diet[2] CTL	PM 25	PM 50	PM 75	PM 100	SEM
BW, g						
d 14	417.73[bc]	387.55[c]	442.09[ab]	397.93[c]	465.47[a]	7.47
d 28	1,448.97[bc]	1,404.75[c]	1,637.43[ab]	1,535.16[abc]	1,694.34[a]	39.57
d 42	2,761.50[b]	2,625.49[b]	2,959.93[ab]	2,796.32[b]	3,215.34[a]	96.70
Feed intake, g						
d 1 to 14	397.46[a]	356.38[abc]	342.11[b]	315.75[c]	366.48[ab]	9.27
d 1 to 28	2,918.82[a]	2,679.36[a]	2,350.10[b]	2,177.07[b]	2,324.17[b]	65.16
d 1 to 42	6,307.71[a]	6,017.03[a]	5,209.04[b]	4,912.05[b]	5,128.70[b]	167.41
Feed conversion, g feed/g gain						
d 1 to 14	0.95[a]	0.92[a]	0.78[b]	0.79[b]	0.79[b]	0.02
d 1 to 28	2.03[a]	1.91[a]	1.44[b]	1.42[b]	1.38[b]	0.05
d 1 to 42	2.30[a]	2.31[a]	1.78[b]	1.76[b]	1.60[b]	0.07

[1] Values are the means of 8 observations per diet.

[2]CTL: corn-based diet; PM 25, PM 50, PM 75 and PM 100: a similar diet in which corn was replaced by 25 %, 50 %, 75 % or 100 % with PM respectively.

[a-c] Values with different superscripts within the same row are different.

Table 3.4. Effects of dietary treatments on jejunal digesta viscosity, ileal DM concentrations and apparent ileal digestibility of CP in broiler chickens[1]

Age	Diet[2]					SEM
	CTL	PM 25	PM 50	PM 75	PM 100	
Viscosity, cP						
d 14	5.45	5.46	5.32	5.27	5.22	0.09
d 28	5.58	5.34	5.39	5.42	5.39	0.11
d 42	5.61	5.51	5.66	5.64	5.58	0.08
Ileal DM, %						
d 14	18.15	18.46	18.07	17.93	18.99	0.48
d 28	15.93[b]	17.53[ab]	18.31[ab]	19.44[a]	19.45[a]	0.80
d 42	15.94[b]	16.67[b]	18.87[a]	18.13[a]	19.26[a]	0.35
CP digestibility, %						
d 14	0.76	0.80	0.76	0.74	0.77	0.02
d 28	0.73	0.71	0.73	0.73	0.76	0.02
d 42	0.78	0.80	0.77	0.79	0.81	0.01

[1] Values are the means of 8 observations per diet.

[2]CTL: corn-based diet; PM 25, PM 50, PM 75 and PM 100: a similar diet in which corn was replaced by 25 %, 50 %, 75 % or 100 % with PM respectively.

[a-b]Values with different superscripts within the same row are different.

Table 3.5. Effects of dietary treatments on villi height, width and surface area of the jejunum of broiler chickens[1]

	Villi height (µm)		Villi width (µm),		Surface area (mm^2)	
Diet[2]	d 28	d 42	d 28	d 42	d 28	d 42
CTL	1,696	1,988	175.28	181.64	0.93	1.22
PM 25	1,822	1,832	243.82	214.76	1.40	1.24
PM 50	2,159	2,003	239.25	225.48	1.64	1.43
PM 75	1,967	2,016	276.54	204.81	1.74	1.31
PM 100	1,937	2,156	195.51	237.54	2.30	1.54
SEM	92.12	86.68	23.67	15.75	0.16	0.10

[1] Values are the means of 8 observations per diet.

[2]CTL: corn-based diet; PM 25, PM 50, PM 75 and PM 100: a similar diet in which corn was replaced by 25 %, 50 %, 75 % or 100 % with PM respectively.

Table 3.6. Effects of dietary treatments on *E. coli*, *Lactobacilli* and *Bifidobacteria* concentrations in the ceca of broiler chickens[1]

Age	Diet[2] CTL	PM 25	PM 50	PM 75	PM 100	SEM
E. coli, log_{10} cfu/g						
d 14	7.17	6.88	6.82	6.68	6.33	0.25
d 28	9.61[a]	9.52[a]	9.59[a]	8.88[b]	8.92[b]	0.12
d 42	9.96	9.89	9.81	9.70	9.92	0.09
Lactobacilli, log_{10} cfu/g						
d 14	7.69[b]	8.74[a]	7.98[ab]	7.63[b]	8.12[ab]	0.21
d 28	8.62[ab]	8.84[a]	8.74[a]	8.57[ab]	8.05[b]	0.14
d 42	9.03	8.76	9.04	8.53	8.55	0.13
Bifidobacteria, log_{10} cfu/g						
d 14	2.53	2.38	2.23	2.16	2.24	0.16
d 28	3.53	3.47	3.48	3.27	3.11	0.15
d 42	2.95	3.08	3.03	2.90	2.97	0.15

[1] Values are the means of 8 observations per diet.

[2]CTL: corn-based diet; PM 25, PM 50, PM 75 and PM 100: a similar diet in which corn was replaced by 25%, 50%, 75% or 100% with PM respectively.

[a-b] Values with different superscripts within the same row are different.

Preface to Chapter 4

Chapter 4 comprises a manuscript, ready to be submitted for publication in the journal of *Poultry Science*. All literature cited in this chapter is listed in the 'References' section at the end of the thesis.

Results from Experiment 1, presented in Chapter 3, demonstrate that total replacement of corn with the Canadian pearl millet in broiler diets resulted in significant improvements in growth and feed efficiency, and that there were no detrimental effects on digesta viscosity, development of morphological structures, and *E. coli*, *Lactobacilli* and *Bifidobacteria* populations of the intestines.

The aim of Experiment 2, described in Chapter 4, was to confirm whether totally replacing corn with Canadian pearl millet would lead to significant improvements in growth performance. Additionally, we investigated whether enzyme supplementation to pearl millet diets would help to further maximize growth performance. Finally, we were interested to determine whether pearl millet's higher crude protein and amino acids may represent the responsible nutritional factors for the observed improvements in growth parameters.

CHAPTER 4

Growth performance, ileal digestibility, and intestinal development and microbial populations of broiler chickens fed corn- or Canadian pearl millet-based diets with or without exogenous enzymes[1]

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Growth performance, ileal digestibility, and intestinal development and microbial populations of broiler chickens fed corn- or Canadian pearl millet-based diets with or without exogenous enzymes

4.1 ABSTRACT

An experiment was conducted to investigate the effects of replacing corn with pearl millet in broiler diets, alone or in combination with exogenous enzymes, on growth performance, jejunal villus development, ileal CP and amino acid digestibility, and cecal microbial populations. One hundred and sixty 1-d-old male Ross 508 broilers (8 cage replicates) were randomly allocated to one of the following dietary treatments: 1) a standard corn-soybean meal diet (CTL); 2) a pearl millet-soybean meal diet (PM); 3) diet 1 + exogenous enzymes (CE); and 4) diet 2 + exogenous enzymes (PE). PM and PE contained less soybean meal because pearl millet grains were richer in CP and amino acids than corn. All diets were isonitrogenous and isocaloric. Bodyweight and feed intake were recorded weekly over 35 d. At d 21 and 35, 8 birds per treatment were euthanized for sample collection and analyses. Feed conversion was improved ($P < 0.05$) with pearl millet- than corn-based diets. Ileal digestibility of CP and amino acids was similar between PM and CTL, but increased ($P < 0.05$) when birds were fed CE than CTL. In comparison to PM-fed birds, PE improved ($P < 0.05$) CP digestibility but not for amino acids. Villus was longer ($P < 0.05$) in birds fed PM than CTL or CE at d 35. Similarly, at d 35, *Lactobacilli* loads were higher ($P < 0.05$) in birds fed PM than CTL, and in those fed PE than CTL or CE. Therefore, substituting corn for pearl millet in broiler diets can improve production parameters, and *Lactobacilli* populations and villus development of the intestines whereas exogenous enzymes supplementation can increase CP digestibility.

(**Key words**: pearl millet, enzymes, villus height, digestibility, broilers)

4.2 INTRODUCTION

Corn, the most utilized grain in formulating broiler diets with inclusion rates exceeding 50%, contributes about 65% of broiler ME requirements due to its high ME contents. However, over the past years, corn has been marketed at high market prices due to its preferential diversion towards human consumption and corn-ethanol industry. The unprecedented irregular corn supply and economic challenge that has known the poultry industry has triggered strong interests to curb down reliance on corn. Previously, we discussed the detrimental effects of replacing corn with wheat, barley or rye on broiler performance, and intestinal digesta viscosity, nutrient digestibility, morphological development and microflora (Baurhoo et al., in press). Most importantly, we demonstrated that a new hybrid Canadian pearl millet variety (CGPMH-90, Agriculture Environmental Renewal Canada Inc.; AERC Inc., Ontario, Canada) could totally replace corn with significant improvements in BW gain (3.22 kg v/s 2.66 kg) and feed conversion ratio (1.6 v/s 2.3; **FCR**) at 42 d without affecting the above-mentioned intestinal parameters.

The mechanistic underlying improvements in growth and feed conversion when feeding broilers diets formulated with pearl millet than corn still remains unknown. In comparison to corn, pearl millet grains contain comparable ME levels (3,093 vs 3,355 kcal/kg) but higher CP (12.88 vs 6.49%) and amino acid concentrations, especially lysine, methionine, threonine and arginine that are most limiting to poultry (Yin et al., 2002; Baurhoo et al., in press). Therefore, we were interested in determining whether pearl millet's higher CP and amino acids may be responsible for improvements in growth parameters. To that end, we formulated PM-based diets containing less soybean meal (SBM) to be iso-nitrogeneous compared to corn-based diets. Although costly, SBM is widely considered as the major source of highly digestible CP and amino acids with inclusion rates above 30% in broiler diets. It is worth mentioning that higher digestibilities of CP and amino acids, including lysine, arginine, threonine, valine and isoleucine, have been reported in pigs fed pearl millet- than corn-based diets (Adeola and Orban, 1995), but the study did not evaluate effects on growth performance.

There is considerable evidence that supplementation of exogenous enzymes to corn-based diets caused significant improvements in FCR in broilers most probably due to increased nutrient digestibility, including DM, CP, ME and starch (Kocher et al., 2003; Meng and Slominski., 2005; Cowieson and Ravindran, 2008; Zhou et al., 2009). Therefore, we also hypothesized that enzyme supplementation to pearl millet-based diets would contribute to maximize nutrient digestibility and utilization, thereby leading to further improvements in performance. This is the first study evaluating the effects of enzyme supplementation to diets formulated with Canadian pearl millet on broiler performance. Finally, since our previous study was the first to report that the Canadian pearl millet can fully replace corn in broiler diets and causing significant improvements in growth (Baurhoo et al., in press), we were here interested in confirming these findings.

Hence, the objectives of this study were to compare the effects of pearl millet- and corn-based diets together with or without enzyme supplementations on growth, ileal CP and amino acid digestibilities, and intestinal morphological development and microbial populations in broilers.

4.3 MATERIALS AND METHODS

4.3.1 Bird Management

One hundred and sixty male Ross 508 broilers obtained from a local commercial hatchery (Couvoir Simentin, Mirabel, Quebec, Canada) were grown in cages over a 35-d experimental period. Eight cage replicates housing five birds each were randomly assigned to 1 of 4 dietary treatments. Birds were housed in an environmentally controlled room in which temperature was gradually decreased from 32 to 24°C by 0.5°C daily under a 20L:4D lighting cycle. Protocols involved in this study were approved by the Animal Care Committee of McGill University. Birds were group weighed by cage and feed intake was determined at weekly intervals.

4.3.2 Experimental diets

The study was conducted as 2 x 2 factorial arrangements of dietary treatments. The 4 experimental diets included: 1) a standard corn-soybean meal diet (**CTL**); 2) a similar diet in which corn was totally replaced with the Canadian pearl millet (**PM**; AERC Inc.); 3) diet 1 + enzymes (**CE**); 4) diet 2 + enzymes (**PE**). Enzymes blend used included Vegpro (50g/T of feed; Alltech Inc., Nicholasville, KY) and Robavio Excel AP (100g/T of feed; Adisseo Canada Inc., ON, Canada). Vegpro is a multi-enzyme complex that contains 7,500 units of protease and 44 units of cellulase per gram with side activities of pentosanase, α-galactosidase and amalyse. Rovabio Excel AP activities as reported by the supplier are 22,000 visco units/g (equivalent to 1,400 units/g) for endi-1,4-*β* xylanase and 2,000 units/g for endo-1,3(4) *β*-glucanase. Birds had *ad-libitum* access to experimental diets and water. Mashed diets were formulated to be isoenergetic, isonitrogenous and to meet or exceed the minimum requirements for macro- and micronutrients (Table 4.1).

Finally, chromic oxide (3g/kg) was incorporated in all diets as an indigestible marker for ileal nutrient digestibility.

4.3.3 Sample Collections

At 21 and 35 d of age, one bird per treatment cage (n = 8/treatment) was randomly selected, weighed and sacrificed by electrical stunning and bleeding of the carotid artery. The jejunum (2 cm from the end of duodenum to Meckel's diverticulum), ileum (Meckel's diverticulum up to 40 mm above the ileo-cecal junction) and both ceca were carefully excised and collected.

4.3.4 Gut Digesta Viscosity

The jejunal contents of respective treatment birds were carefully hand-stripped into a 15 mL centrifuge tube and viscosity immediately measured as described (Baurhoo et al., in press). Briefly, 2 g of digesta were centrifuged (12,000 g, 10 mins). After the supernatant was gently mixed by shaking, a 1 mL volume was used in an advanced rheometer (Model AR 2000EX; TA Instruments, DE) fitted with a CP40 cone at 40°C and shear rate varying from 5 to 500 s^{-1}. Readings were taken at 42 s^{-1} and recorded in cPs.

4.3.5 Gut histomorphology

A 1-cm segment of the jejunum (n=8/treatment) was washed in physiological saline solution, fixed in 10 % buffered formalin solution, washed thrice in 0.1 M phosphate buffered saline (pH 7.4) for 15 min each, dehydrated using increasing ethanol concentrations at 70 % (overnight), 80% (1 hr), 90% (1 hr) and 100% (1 hr over 3 consecutive times), and then embedded in paraffin. Using a microtome, each tissue sample was cross-sectioned with a thickness of 5-μm (3 per sample), placed on a glass slide, and stained with hematoxylin and eosin. A phase contrast microscope with integrated image analysis NIS-Element BR v. 2.3 software (NIKON DXM 1200c, Nikon Corporation, Tokyo, Japan) was used to measure villus height and width. Villus height was defined as distance measured from the tip of the villus to the top of the lamina propria whereas villus width was measured at the basal and apical ends. Villus surface area was calculated using the formula (2π)(villus width / 2)(villus height) (Sakamoto et al., 2002). Ten replicate measurements per treatment birds were used in statistical analysis.

4.3.6 Microbiological analysis

Fresh cecal samples of treatment birds (n=8/treatment) were diluted 10-fold by weight in buffered peptone water (Fisher Scientific, Ontario, Canada). Samples were mixed by vortexing and serially diluted in 0.85% sterile saline solution and used for enumeration of *Lactobacilli* and *E. coli*. All microbiological analyses were performed in duplicate and the average values used in statistical analysis. *Lactobacilli* were enumerated using Man, Rogosa and Sharpe agar (Fisher scientific) whereas *E. coli* was assayed using Rapid E. coli 2 agar (BioRad Laboratories, Missisauga, Ontario, Canada). Agar plates were incubated at 37°C for 24 or 48 after which bacterial colonies were counted.

4.3.7 Ileal Nutrient Digestibility

Ileal digesta from 2 birds per treatment cage (n = 16/treatment) were pooled to obtain enough samples for nutrient digestibility analysis. Ileal digesta from respective bird groups were freeze dried whereas diet samples were dried overnight in a forced air oven at 70°C. Then, all samples were ground to pass through a 0.5-mm sieve and stored in airtight containers at –20°C until analysis. All analyses were done in duplicates. DM and organic matter were determined according to the standard methods of the AOAC (1990). Crude protein (N x 6.25) was determined using an N Analyzer (model FP 428, Leco Corp., St Joseph, MI). Samples for amino acid analysis were prepared by acid hydrolysis according to the method of AOAC (1990; method 982.30) and as described by Woyengo et al. (2010). Briefly, about 100 mg of each sample was digested in 4 mL of 6 *N* HCl for 24 h at 110°C, neutralized with 4 mL of 25% (wt/vol) NaOH, and cooled to room temperature. Then, the mixture was brought to a final volume of 50-mL with sodium citrate buffer (pH 2.2) and analyzed using an amino acid analyzer (Sykam, Eresing, Germany). Chromic oxide was determined using the methods of Fenton and Fenton (1979) and as described by Baurhoo et al. (in press). Apparent ileal digestibility (AID) coefficients for DM, organic matter, CP and amino acids were calculated using the following equation:

$$AID\ (\%) = 1 - \left[\left({Cr_i}/{Cr_0}\right) \times \left({NT_0}/{NT_i}\right)\right]$$

where Cr_i is the concentration of chromic oxide in the feed, Cr_o is the concentration of chromic oxide in the digesta, NT_0 represents the concentration of chromic oxide in the digesta and NT_i is the concentration of chromic oxide in the feed.

4.3.8 Statistical Analysis

Data were analyzed as a two-way ANOVA and a 2 x 2 factorial arrangement to determine the main effects of diet (grain type) and enzyme, and their interaction effects by using the MIXED procedure of SAS (SAS Institute, 2003). Differences among treatment means were tested using Scheffe's Multiple Comparison *t*-test and statistical significance declared at $P < 0.05$. Microbiological samples were subjected to base-10 logarithm transformations before statistical analysis.

4.4 RESULTS

4.4.1 Bodyweight, feed conversion ratio and feed intake

Table 4.2 shows that the main effect of grain type had a major influence on BW, FI and FCR throughout the study (d 1-35). Overall, birds consumed less of the pearl millet-based diet, but had greater BW and lower FCR in comparison to birds fed corn diets. But, the enzyme's main effect did not alter BW and FCR. There was also no significant interaction effect between grain type and enzyme supplementation; BW and FCR were similar with or without enzyme supplementation within grain type at d 7, 21 and 35. Interestingly, feed conversion was significantly lowered among birds fed PM and PE than CTL and CE diets. At d 7 and 21, higher BW was recorded when birds were fed PM and PE in comparison to those fed CE but not CTL. But, at d 35, birds were heavier when fed PE than CE or CTL.

However, at d 7 and 21, FI was significantly reduced by the main effect of enzyme supplementation. Additionally, at d 7, 21 and 35, our FI data shows interaction effects between enzyme supplementation and corn only. FI was reduced when birds were fed CE than CTL; but similar effects did not occur between PM- and PE-fed birds.

4.4.2 Viscosity and AID of Dry Matter, Crude Protein and Organic Matter

Viscosity of jejunal digesta was not significantly affected by grain type, enzyme supplementation or an interaction between these two factors (Table 4.3). At d 21 and 35, enzyme supplementation had a major overall effect on nutrient digestibility in that to consistently increase AID of DM, OM and CP (Table 4.3). But, nutrient digestibility was not influenced by grain type, except for AID of OM at d 35 which was higher for PM-based diets. The positive effects of enzyme on nutrient digestibility reflected in the interaction effect between enzyme and grain type; AID of DM, OM and CP were increased upon addition of enzymes to CTL or PM diet throughout the study, except for AID of DM and OM between PM and PE at d 21.

4.4.3 Ileal Amino Acids Digestibility

Overall, enzyme supplementation significantly increased AID of all amino acids throughout the experimental period (Tables 4.4 and 4.5). But, with the exception that glycine and arginine AID were lower in pearl millet-based diets than corn-diets at d 21, grain type had no major effects on AID of amino acids throughout the study. There was no difference in AID of all amino acids between birds fed the CTL and PM or CE and PE diets at d 21 and 35. However, in comparison to the CTL diet, enzyme supplementation (CE) significantly increased digestibility of all amino acids at d 21 and 35, except for glutamine, proline, valine, phenylalanine, lysine and arginine which were not different at d

21. On the other hand, similarity in amino acids digestibility between PM and PE at both d 21 and 35 indicate that enzyme had no interaction effect with pearl millet diet.

4.4.4 Enumeration of *Lactobacilli* spp. and *E. coli* in the ceca

There were no significant effects of grain types, enzyme addition or interactions between the two factors on *Lactobacilli* and *E. coli* populations at d 21 (Table 4.6). Similar observations were recorded for *E. coli* populations at d 35. Our findings also indicated that irrespective of enzyme supplementation, pearl millet-based diets significantly increased viable counts of *lactobacilli* at d 35. But, such effect did not occur due to enzyme supplementation. Finally, at d 35, birds fed PM and PE had larger *Lactobacilli* population that CTL-fed birds.

4.4.5 Histomorphology

At d 35, villi height was significantly increased due to main effects of pearl millet than corn (Table 4.7). At same bird age, longer villi were observed among birds fed the PM diet than CTL or CE. But, villi height was not altered due to enzyme supplementation. Cereal type, enzyme or interactions between both factors did not affect villi width and villi surface area throughout the study.

4.5 DISCUSSION

Findings of this study are in agreement with our previous experiment (Baurhoo et al., in press) confirming that pearl millet could fully replace corn in broiler diets leading to significant improvements in FCR. Growth was comparable despite reduced consumption of the PM diet. In another study with broilers, Davis et al. (2003) observed BW improvements when 33% of dietary corn was replaced with pearl millet. However, BW and feed efficiency were not altered when replacing 5 to 75% of dietary corn with pearl millet in broilers (Davis et al., 2003; Hildago et al., 2004; Manwar and Mandal, 2009) and pigs (Lawrence et al., 1995).

Additionally, we observed that addition of enzymes to corn- or pearl millet-based diets had no major effects on BW and FCR. Our findings are in agreement with some reports indicating that enzyme supplementation to corn-based diets did not alter BW and FCR (West et al., 2007; Aftab et al., 2009; Madrid et al., 2010). But, several other authors reported significant improvements in BW and FCR due to enzyme supplementation to corn-diets (Zanella et al., 1999; Kocher et al., 2003; Owens et al., 2008). Taken together, all these findings reveal inconsistency in the effects of enzyme supplementation to corn diets on broiler performance. Research studies about evaluating effects of enzyme supplementation to pearl millet diets on broiler performance are limited. But, Manwar and Mandal (2009) observed that exogenous enzymes had no effect on BW and FCR when 25% dietary corn was replaced with pearl millet.

The only positive effect of enzymes on performance parameters that occurred was reduced consumption of CE than the CTL diet. Interestingly, a reduction in CE intake was not accompanied by depressed growth. Exogenous enzymes are known to cause enzymatic breakdown of non-starch polysaccharides (NSP) and release of trapped nutrients causing improvements in efficiency of feed utilization. Given that enzymes failed to also reduce consumption of PE than the PM diet, our findings led us to believe that PM contained less NSP (soluble + insoluble) in comparison to the CTL diet. But, as later herein discussed, our results about digesta viscosity demonstrated that soluble NSP contents were similar between PM and CTL diets. It is, therefore, conceivable to believe that PM contained less insoluble type of NSP than the CTL diet. Higher insoluble NSP might be attributed to PM's reduced SBM inclusion rates. SBM is reported to contain higher NSP (18.77 % DM basis) concentrations than pearl millet grains (2.2% DM basis) (Englyst, 1989; Irish and Balnave, 1993).

We previously reported similarity in intestinal digesta viscosity between PM and CTL diets in broilers (Baurhoo et al., in press). In this study, we observed unchanged digesta viscosity between PM and CTL diets with or without enzyme supplementation. Intestinal digesta viscosity is a linear function of dietary water-soluble NSP concentrations that bind water of the digesta (Choct and Annison, 1992; Langhout et al., 1999). Therefore, it is most likely that soluble NSP contents were not different between pearl millet- and corn-based diets. It is well documented that wheat (2.4%), barley (4.5%) and rye (4.6%) contain high soluble NSP levels especially β-glucans and arabinoxylans (Englyst, 1989; Choct, 2006), and that inclusion of these grains into poultry diets necessitates enzyme supplementation to alleviate the problems of high viscosity, reduced nutrient utilization and depressed growth (Marquardt et al., 1994; Mathlouthi et al., 2002; Silva and Smithard, 2002; Shirzadi et al., 2010). It appears that corn and pearl millet diets contained relatively low soluble NSP levels. Evidently, traces of soluble NSP in the form of arabinoxylans mostly, have been reported in corn (0.1%) and pearl millet (0.2%) (Choct, 2006).

This is the first study investigating effects of enzyme supplementation to pearl millet-based diets on nutrient digestibility in broilers. Broilers fed the PM or CTL diet had equivalent effects on AID of DM, organic matter, CP and amino acids. These are interesting findings considering that PM was formulated to contain less soybean meal than the CTL diet. But, addition of enzymes to corn (CTL vs CE) or pearl millet (PM vs PE) diets significantly increased DM, organic matter and CP digestibility. Our findings are in agreement with previous reports that enzyme addition to corn-based diets significantly increased nutrient digestibility without having any effect on digesta viscosity (Almirall et al., 1995; Zanella et al., 1999; Garcia et al., 2003; Jia et al., 2009) and growth (Madrid et al., 2010).

Whereas amino acid digestibility was markedly increased upon enzyme supplementation to the CTL diet (CE vs CTL), such effect was not observed in birds fed PE or PM. The higher SBM inclusion rates in corn diets may explain the higher amino acid digestibility among birds fed CE than CTL. We previously discussed that enzymes are effective in digesting SBM's high NSP contents, and that SBM represented the major source of amino acids in CTL and CE diets. On the other hand, despite reduced inclusion rates of soybean meal in PM and PE, pearl millet's higher CP and amino acid contents were successful in sustaining CP and amino acid digestibility. These findings are indicative that pearl millet's higher amino acid contents were highly digestible and available to chickens. In the event that pearl millet would have contained less or poorly digestible amino acids, AID of amino acids as well as broiler growth would majorly have been compromised. CP and essential amino acids are critical for efficient protein utilization and meat deposition (Corzo et al., 2005; Schutte and Jong, 2004).

Feeding broilers PM than the CTL diet also significantly increased intestinal populations of *Lactobacilli*. Establishment of an intestinal population of *Lactobacilli* is generally regarded as a condition of good intestinal health. There is compelling evidence that *Lactobacilli* secrete antimicrobial compounds such as bacteriocins that cause a reduction in the intestinal populations of harmful bacteria (Jin et al., 1996). At this stage, it is difficult to explain the increased *Lactobacilli* loads. But, we previously reported no difference in *Lactobacilli* loads between broilers fed CTL or PM (Baurhoo et al., in press). There were, however, no dietary effects on *E. coli* loads. This was not surprising considering the similarity in intestinal digesta viscosity between PM- and corn-fed birds. The correlation between increased digesta viscosity and increased intestinal concentrations of *E. coli* has previously been reported in broilers (Langhout et al., 1999).

Previous studies have associated increased villi height with increased intestinal populations of *Lactobacilli* (Sieo et al., 2005; Baurhoo et al., 2007; Awad et al., 2009); beneficial bacteria enhanced vascularization and development of the intestinal villi (Stappenbeck et al., 2002). The association between *Lactobacilli* and increased villi height has also been evidenced in this study due to consumption of PM than the CTL diet. Finally, our findings that enzyme supplementation had no effects on *Lactobacilli* populations and villi height are in agreement with those of Owens et al. (2008) and Lü et al. (2009).

In conclusion, replacing corn with pearl millet in broiler diets caused significant improvements in feed efficiency, and increased *Lactobacilli* populations and villus development of the intestines, but had equivalent effects on intestinal digesta viscosity and nutrient digestibility. No major effects of enzyme supplementation were observed in that it improved DM and CP digestibility only. Therefore all these findings, together with the fact that pearl millet diets were formulated to contain less soybean meal, support our hypothesis that improved performance parameters due to pearl millet-based diets are due to pearl millet's higher CP and amino acids contents. It is clear that the Canadian PM can fully replace corn in broiler diets.

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Table 4.1. Composition (%) and calculated analysis (%) of corn and pearl millet basal diets

Item	Corn[1]	Pearl millet[1]
Ingredient		
Corn	52.98	0.00
Pearl millet	0.00	59.17
Soybean meal, 48 % CP	39.47	31.14
Soybean oil	3.30	4.97
DL-Met	0.19	0.17
Lys-HCl	0.02	0.15
Vitamin-mineral premix[2]	0.67	0.67
Monocalcium phosphate	1.52	1.76
Sodium carbonate	0.10	0.10
Limestone	1.23	1.21
Sodium chloride	0.22	0.26
Chromium oxide	0.30	0.40
Calculated analysis		
ME, kcal/kg	3,050.00	3,050.00
CP	22.50	22.50
Crude Fat	5.53	8,40
Lys	1.35	1.35
Met	0.55	0.55
Ca	0.90	0.09
Available P	0.45	0.45

[1]Corn and pearl millet diets were supplemented with Vegpro (50g/T of diet; Alltech Inc., Nicholasville, KY) and Robavio Excel AP (100g/T of feed; Adisseo Canada Inc., ON, Canada). Vegpro is a multi-enzyme complex that contained 7,500 units of protease and 44 units of cellulase per gram with side activities of pentosanase, α-galactosidase and

amalyse. Rovabio Excel AP contained 22,000 visco units/g (equivalent to 1,400 units/g) for endi-1,4-*β* xylanase and 2,000 units/g for endo-1,3(4) *β*-glucanase.

[2]Supplied per kilogram of diet: vitamin A, 6,000 IU; vitamin D, 3,000 IU; vitamin E, 50.21 IU; vitamin B12, 0.03 mg; vitamin K, 2.04 mg; biotin, 0.15 mg; choline, 1,300.5 mg; folic acid, 1.47 mg; niacin, 48.99 mg; panthothenic acid, 13.35 mg; riboflavin, 6.4 mg; thiamine, 2.23 mg; pyridoxine, 3.20 mg; Cu, 12.63 mg; Fe, 22.6 mg; Mn, 108.53 mg; Zn, 108.03 mg; Co, 0.46 mg; I, 0.8 mg; and Se, 0.39 mg

Table 4.2. Effects of dietary treatments on BW, feed intake, and feed conversion of broiler chickens[1]

Item		BW (g/bird)			Feed intake (g/bird)			Feed conversion (g feed/g gain)		
Diet	Enzyme	7 d	21 d	35 d	7 d	21 d	35 d	7 d	21 d	35 d
Corn	No[2]	161.53[ab]	954.89[a]	2322.38[b]	132.49[a]	1221.91[a]	4225.09[a]	0.82[b]	1.28[b]	1.82[b]
	Yes[2]	159.49[b]	877.97[b]	2253.50[b]	130.30[b]	1120.08[b]	3964.14[b]	0.82[b]	1.28[b]	1.76[b]
Pearl millet	No[2]	162.47[a]	992.90[a]	2382.00[ab]	129.34[b]	1096.36[b]	3482.72[c]	0.79[a]	1.11[a]	1.47[a]
	Yes[2]	163.57[a]	1000.70[a]	2491.88[a]	128.79[b]	1078.93[b]	3564.47[c]	0.79[a]	1.08[a]	1.43[a]
Pooled SEM		0.58	18.14	28.27	0.51	11.86	69.33	0.01	0.02	0.04
Main Effects										
Diet	Corn	160.51[y]	916.43[y]	2287.94[y]	131.40[x]	1170.99[x]	4094.61[x]	0.82[y]	1.28[y]	1.79[y]
	Pearl millet	163.02[x]	996.80[x]	2436.94[x]	129.06[y]	1087.64[y]	3523.60[y]	0.79[x]	1.09[x]	1.44[x]
Enzyme	No	162.00	973.90	2352.19	130.91[x]	1159.13[x]	3853.90	0.81	1.19	1.64
	Yes	161.53	939.33	2322.38	129.55[y]	1099.50[x]	3764.31	0.80	1.18	1.60
Pooled SEM		0.41	12.82	39.97	0.36	11.86	49.02	0.01	0.02	0.03

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

[a-c, x-y] Values with different superscripts within the same column are different.

Table 4.3. Effects of dietary treatments on jejunal digesta viscosity, and apparent ileal digestibility of DM, OM and CP in broiler chickens[1]

		Viscosity, cP		Ileal digestibility, %					
				DM		OM		CP	
Diet	Enzyme	d 21	d 35	d 21	d 35	d 21	d 35	d 21	d 35
Corn	No	5.12	4.91	0.72[b]	0.67[b]	0.71[b]	0.68[c]	0.78[b]	0.72[b]
	Yes	5.05	4.82	0.76[a]	0.75[a]	0.75[a]	0.76[a]	0.82[a]	0.83[a]
Pearl millet	No	5.04	4.72	0.72[b]	0.70[b]	0.71[b]	0.72[b]	0.78[b]	0.76[b]
	Yes	5.26	4.75	0.74[ab]	0.76[a]	0.73[ab]	0.77[a]	0.80[a]	0.83[a]
Pooled SEM		0.11	0.09	0.01	0.01	0.01	0.01	0.01	0.01
Main Effects									
Diet	Corn	5.09	4.87	0.74	0.71	0.73	0.72[y]	0.80	0.78
	Pearl millet	5.15	4.74	0.73	0.73	0.72	0.75[x]	0.79	0.79
Enzyme	No	5.08	4.81	0.72[y]	0.69[y]	0.71[y]	0.70[y]	0.78[y]	0.74[y]
	Yes	5.16	4.79	0.75[x]	0.76[x]	0.74[x]	0.77[x]	0.81[x]	0.83[x]
Pooled SEM		0.07	0.06	0.01	0.01	0.01	0.01	0.01	0.01

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

[a-b, x-y] Values with different superscripts within the same column are different.

Table 4.4. Effects of dietary treatments on apparent ileal digestibility (%) of amino acids in broiler chickens at d 21[1]

		Indispensable amino acids									Dispensable amino acids							
Diet	Enzyme	Met	Thr	Val	Ile	Leu	His	Lys	Phe	Arg	Gly	Ala	Cys	Asp	Ser	Pro	Tyr	Glu
Corn	No	0.87^{b}	0.72^{b}	0.81^{ab}	0.81^{b}	0.82^{b}	0.82^{b}	0.83	0.81	0.87	0.75^{b}	0.81^{b}	0.71^{b}	0.78^{b}	0.74^{b}	0.85	0.78^{b}	0.85
	Yes	0.91^{a}	0.78^{a}	0.85^{a}	0.86^{a}	0.86^{a}	0.86^{a}	0.87	0.85	0.89	0.80^{a}	0.85^{a}	0.77^{a}	0.82^{a}	0.81^{a}	0.88	0.83^{a}	0.88
Pearl millet	No	0.87^{b}	0.74^{b}	0.80^{b}	0.82^{ab}	0.82^{b}	0.82^{b}	0.84	0.81	0.86	0.74^{b}	0.80^{b}	0.74^{bc}	0.80^{ab}	0.76^{bc}	0.85	0.80^{ab}	0.85
	Yes	0.89^{ab}	0.76^{ab}	0.81^{ab}	0.82^{ab}	0.84^{ab}	0.84^{ab}	0.85	0.82	0.87	0.76^{b}	0.83^{ab}	0.78^{ac}	0.80^{ab}	0.79^{ac}	0.87	0.80^{ab}	0.87
Pooled SEM		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Main Effects																		
Diet	Corn	0.89	0.75	0.83	0.83	0.84	0.84	0.85	0.83	0.88^{x}	0.78^{x}	0.83	0.74	0.80	0.77	0.87	0.80	0.87
	Pearl millet	0.88	0.75	0.81	0.82	0.83	0.83	0.84	0.82	0.86^{y}	0.75^{y}	0.82	0.76	0.80	0.77	0.86	0.80	0.86
Enzyme	No	0.87^{y}	0.73^{y}	0.80^{y}	0.81^{y}	0.82^{y}	0.82^{y}	0.83^{y}	0.81^{y}	0.86^{y}	0.75^{y}	0.81^{y}	0.73^{y}	0.79^{y}	0.75^{y}	0.85^{y}	0.79^{y}	0.85^{y}
	Yes	0.90^{x}	0.77^{x}	0.83^{x}	0.84^{x}	0.85^{x}	0.85^{x}	0.86^{x}	0.84^{x}	0.88^{x}	0.78^{x}	0.84^{x}	0.78^{x}	0.81^{x}	0.80^{x}	0.87^{x}	0.81^{x}	0.87^{x}
Pooled SEM		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

[a-b, x-y] Values with different superscripts within the same column are different.

Table 4.5. Effects of dietary treatments on apparent ileal digestibility (%) of amino acids in broiler chickens at d 35[1]

		Indispensable amino acids									Dispensable amino acids							
Diet	Enzyme	Met	Thr	Val	Ile	Leu	His	Lys	Phe	Arg	Gly	Ala	Cys	Asp	Ser	Pro	Tyr	Glu
Corn	No	0.81^{b}	0.65^{b}	0.77^{b}	0.78^{b}	0.79^{b}	0.80^{b}	0.77^{b}	0.78^{b}	0.84^{b}	0.72^{b}	0.76^{b}	0.77^{b}	0.75^{b}	0.69^{b}	0.83^{b}	0.77^{b}	0.83^{b}
	Yes	0.90^{a}	0.81^{a}	0.87^{a}	0.87^{a}	0.87^{a}	0.88^{a}	0.88^{a}	0.87^{a}	0.90^{a}	0.82^{a}	0.86^{a}	0.82^{a}	0.84^{a}	0.82^{a}	0.89^{a}	0.86^{a}	0.89^{a}
Pearl millet	No	0.84^{ab}	0.70^{ab}	0.79^{bc}	0.80^{bc}	0.80^{bc}	0.81^{b}	0.81^{ab}	0.80^{bc}	0.85^{b}	0.73^{b}	0.80^{ab}	0.80^{ab}	0.77^{bc}	0.73^{ab}	0.84^{ab}	0.79^{ab}	0.84^{ab}
	Yes	0.91^{a}	0.78^{a}	0.84^{ac}	0.85^{ac}	0.85^{ac}	0.86^{ab}	0.85^{ab}	0.84^{ac}	0.88^{ab}	0.78^{ab}	0.85^{a}	0.81^{a}	0.82^{ac}	0.79^{ab}	0.88^{a}	0.84^{ab}	0.88^{a}
Pooled SEM		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Main Effects																		
Diet	Corn	0.86	0.73	0.82	0.83	0.83	0.84	0.83	0.83	0.87	0.77	0.81	0.79	0.80	0.76	0.86	0.81	0.86
	Pearl millet	0.88	0.74	0.82	0.83	0.83	0.83	0.83	0.82	0.87	0.75	0.83	0.76	0.79	0.76	0.86	0.81	0.86
Enzyme	No	0.83^{y}	0.67^{y}	0.78^{y}	0.79^{y}	0.80y	0.80^{y}	0.79^{y}	0.79^{y}	0.85^{y}	0.72^{y}	0.78^{y}	0.74^{y}	0.76^{y}	0.71^{y}	0.83^{y}	0.78^{y}	0.83^{y}
	Yes	0.91^{x}	0.79^{x}	0.85^{x}	0.86^{x}	0.86^{x}	0.87^{x}	0.87^{x}	0.85^{x}	0.89^{x}	0.80^{x}	0.86^{x}	0.82^{x}	0.83^{x}	0.81^{x}	0.88^{x}	0.85^{x}	0.88^{x}
Pooled SEM		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

[a-b, x-y] Values with different superscripts within the same column are different.

Table 4.6. Effects of dietary treatments on *Lactobacilli* and *E. coli* concentrations ($\log_{10}$ cfu/g) in the ceca of broiler chickens[1]

		Lactobacillus		*E. coli*	
Diet	Enzyme	d 21	d 35	d 21	d 35
Corn	No	8.31	8.10^{c}	8.15	8.07
	Yes	8.93	8.38^{bc}	8.05	8.22
Pearl millet	No	9.04	8.94^{ab}	7.97	8.53
	Yes	9.36	9.34^{a}	7.91	7.77
Pooled SEM		0.30	0.17	0.26	0.28
Main Effects					
Diet	Corn	8.62	8.24^{y}	8.10	8.15
	Pearl millet	9.20	9.14^{x}	7.94	8.15
Enzyme	No	8.67	8.52	8.06	8.30
	Yes	9.15	8.86	7.97	8.00
Pooled SEM		0.21	0.13	0.18	0.20

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

Table 4.7. Effects of dietary treatments on villi height, width and surface area of the jejunum of broiler chickens[1]

		Villi height (µm)		Villi width (µm)		Villi surface area (mm^2)	
Diet	Enzyme	d 21	d 35	d 21	d 35	d 21	d 35
Corn	No	1789.64	1715.63^{b}	208.87	210.52	1.18	1.13
	Yes	2002.42	1662.55^{b}	180.50	183.45	1.13	0.95
Pearl millet	No	1930.65	2037.27^{a}	194.19	205.56	1.17	1.30
	Yes	1958.84	1989.46^{ab}	153.09	176.28	0.94	1.10
Pooled SEM		51.46	70.85	13.75	21.52	0.08	0.10
Main Effects							
Diet	Corn	1896.03	1689.09^{y}	194.69	196.99	1.15	1.04
	Pearl millet	1944.75	2013.36^{x}	173.64	190.92	1.06	1.20
Enzyme	No	1860.15	1876.45	201.53	208.04	1.18	1.22
	Yes	1980.63	1826.01	166.80	179.87	1.04	1.02
Pooled SEM		39.30	46.86	10.50	14.23	0.06	0.07

[1] Values are the means of 8 observations per diet.

[2] Dietary treatments: CTL: corn-based diet; CE: diet 1 + enzymes; PM: pearl millet-based diet; and PE: diet 3 + enzyme, respectively.

[a-b] Values with different superscripts within the same column are different.

CHAPTER 5:

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Results of this research demonstrate that a total replacement of corn with the Canadian pearl millet significantly improved efficiency of feed utilization for growth or meat deposition in broilers. In comparison to birds fed the CTL diet, feeding broilers PM 100 increased growth in experiment 1; but bodyweight remained unchanged in experiment 2. When fed the PM 100 diet, broilers grew faster when compared to those fed PM 25, PM 50 or PM 75.

Broilers fed PM 100 or CTL had equivalent effects on intestinal digesta viscosity and nutrient (dry matter, CP and amino acid) digestibility. Additionally, in experiment 1, PM 100 and CTL had similar effects on morphological development (villus height, villus width and villus surface area) and concentrations of bacteria (*E. coli*, *Lactobacilli* and *Bifidobacteria*) of the intestines. But, in comparison to the CTL diet, PM 100 increased villi height and *Lactobacilli* populations in experiment 2.

Addition of exogenous enzymes to pearl millet diet (PE) increased intestinal *Lactobacilli* populations when compared to birds fed CTL or CE. Ileal digestibility of CP and amino acids were increased when birds were fed CE than CTL. But, in comparison to PM 100 fed birds, PE increased CP digestibility but not for amino acids.

Therefore, findings of this research demonstrate that the Canadian PM can fully replace corn in broiler diets leading to significant improvements in growth parameters, and increased *Lactobacilli* populations and villus development of the intestines. It appears that enzyme supplementation to pearl millet diets may not be needful since PE failed to improve growth parameters. Finally, pearl millet diets require less soybean meal during diet formulation which may help in reducing feed costs.

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