University of Alberta

Factors affecting variation in nutrient availability of feed ingredients for broiler chickens

by

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Abstract

Nutritive values of feedstuffs used in broiler chicken rations vary. It is important to not only reduce these variations through approaches such as using exogenous enzymes, but also to predict them accurately and rapidly. These efforts can result in formulating more balanced diets, thus allowing optimal animal performance.

Corn, wheat, barley, and field pea samples were evaluated in several in vivo studies to better understand variations in their nutritive values. Several commercial enzyme products were used to determine their effects on reducing variations in energy and amino acid digestibility of corn-, wheat-, or triticale-soy diets. The effectiveness of an in vitro digestibility technique in predicting variations in AME value of wheat and triticale samples was also examined.

The in vivo studies showed variations in availability of nutrients of feedstuffs. However, the extent of these variations was not the same and corn samples were less variable compared to others.

Inclusion of enzymes (xylanase; xylanase, amylase, and protease; xylanase and β-glucanase) in corn-soy diets had transient effects on apparent ileal digestible energy and digestibility of crude protein and amino acid of some of the diets, although enzyme treatments had no effects on performance variables. Supplementing wheat- and triticale-soy diets with a mixture of xylanase, amylase, and protease increased the AME value of wheat and triticale samples, however, the enzyme product had small impact on reducing variations in AME value among the samples.

Measuring physical characteristics did not accurately predict nutritive values of feedstuffs. Chemical characteristics were, to some extent, more relevant. The in vitro digestibility method accurately predicted AME of tested wheat and triticale samples. Addition of several chemical characteristics values into the equation increased the accuracy of prediction of the AME. However, the in vitro method was not able to predict response of a mixture of xylanase, amylase, and protease on the in vivo AME of wheat and triticale samples.

Variations existed in nutritive values of wheat, barley and field pea samples. The in vitro digestibility technique can be an important step in further developments with respect to evaluation of the quality of wheat samples for broiler chickens.

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Table of Contents

Chapter 1: Review of literature 1

1.1 Introduction 1

1.2 Variations in energy availability in wheat 2

1.2.1 Wheat cultivar 3

1.2.2 Environment-related factors 4

1.2.3 Anti-nutritional factors 4

1.2.3.1 NSP and AME values 5

1.2.4 Starch content 6

1.2.5 Wheat storage 7

1.2.6 Animal-related factors 8

1.3 Prediction of variations in nutrient availability of wheat 9

1.3.1 Physical characteristics 10

1.3.2 Chemical analyses 11

1.3.3 In vivo digestibility 11

1.3.4 In vitro digestibility 12

1.3.4.1 Important considerations for developing an in vitro digestibility technique 14

1.3.4.2 Accuracy of in vitro methods 18

1.3.4.3 Use of in vitro digestibility methods 19

1.3.4.4 Application of in vitro digestibility results 21

1.3.5 Near infrared reflectance spectroscopy (NIRS) 21

1.3.5.1 Basic concept ... 21

1.3.5.2 NIRS applications ... 22

1.3.5.3 NIRS limitations ... 23

1.4 The future of feed quality evaluation ... 24

1.5 Conclusions ... 24

1.6 Hypotheses and objectives ... 25

1.7 References ... 27

Chapter 2: Effects of corn source and exogenous enzyme products in broiler chicken diets. Growth performance and apparent ileal digestibility of energy. ... 48

2.1 Introduction ... 48

2.2 Materials and methods ... 50

2.2.1 Experimental design and diets ... 50

2.2.2 Growth performance and digestibility measurements ... 53

2.2.3 Chemical analyses ... 53

2.2.4 Statistical analyses ... 54

2.3 Results ... 54

2.3.1 Growth performance variables ... 55

2.3.2 Digestibility measures ... 55

2.4 Discussion ... 56

2.5 References ... 62

Chapter 3: Effects of corn source and exogenous enzyme products in broiler chicken diets. Apparent ileal digestibility of crude protein and amino acids ... 80

3.1 Introduction 80

3.2 Materials and methods 82

3.2.1 Experimental design and diets 82

3.2.2 Digestibility measurements 83

3.2.3 Chemical analyses 84

3.2.4 Statistical analyses 85

3.3 Results 85

3.4 Discussion 87

3.5 References 91

Chapter 4: Prediction of energetic value of wheat and triticale in broiler chicks: A chick bioassay and an in vitro digestibility technique 104

4.1 Introduction 104

4.2 Materials and methods 106

4.2.1 Test samples 106

4.2.2 Chick bioassay 107

4.2.3 Chemical analyses 108

4.2.4 In vitro digestibility technique 110

4.2.5 Statistical analyses 112

4.3 Results 113

4.3.1 Physico-chemical characteristics of test samples 113

4.3.2 Growth performance variables 113

4.3.3 In vivo digestibility 114

4.3.4 In vitro digestibility 115

4.4 Discussion 116

4.4.1 Physico-chemical characteristics of test samples.................................... 116

4.4.2 Growth performance variables 117

4.4.3 In vivo digestibility 118

4.4.4 In vitro digestibility.. 121

4.5 Conclusions 123

4.6 References 124

Chapter 5: Prediction of response to an NSP-degrading enzyme product on AME of wheat and triticale samples using an in vitro digestibility technique… ……………………………………………………………………………141

5.1 Introduction 141

5.2 Materials and methods 142

5.2.1 Test samples 142

5.2.2 Chick bioassay 143

5.2.3 Growth performance and digestibility measurements 145

5.2.4 Chemical analyses 145

5.2.5 In vitro digestibility technique 147

5.2.6 Statistical analyses.. 147

5.3 Results 148

5.3.1 Physico-chemical characteristics of test samples.................................... 148

5.3.2 Growth performance variables 149

5.3.3 In vivo digestibility 149

5.3.4 Relationship of test sample AME with other characteristics 150

5.3.5 In vitro digestibility 151

5.4 Discussion 151

5.5 References 155

Chapter 6: Evaluating variations in nutrient availability of field peas and barley samples for broiler chickens: A chick bioassay 170

6.1 Introduction 170

6.2 Materials and methods 172

6.2.1 Field pea and barley samples 172

6.2.2 Chick bioassays 173

6.2.3 Growth performance and digestibility measurements 174

6.2.4 Chemical analyses 175

6.2.5 Statistical analyses 177

6.3 Results 178

6.3.1 Physico-chemical characteristics of field pea samples 178

6.3.2 Physico-chemical characteristics of barley samples 179

6.3.3 Growth performance variables 180

6.3.3.1 Field pea study 180

6.3.3.2 Barley study 181

6.3.4 Digestibility of nutrients and energy 181

6.3.4.1 Field pea study 181

6.3.4.2 Barley study 182

6.4 Discussion 184

6.5 Conclusions 188

6.6 References 189

Chapter 7: General discussion 209

7.1 Future directions 214

7.2 References 215

List of Tables

Table 1.1 Variations in AME values of wheat samples reported in different studies…………………………………………………………………………….45

Table 1.2 Factors causing variations in AME values of wheat samples………...46

Table 1.3 Prediction of AMEn of ingredients or complete diets through *in vitro* digestibility techniques in poultry……………………………………………..47

Table 2.1 Chemical analyses of corn samples (%) - DM basis………………....70

Table 2.2 Composition of positive and negative control diets (kg/tonne)……….71

Table 2.3 Analyzed chemical composition of the experimental diets (%) - DM basis……………………………………………………………………………….72

Table 2.4 Recovery of xylanase, amylase, and protease activities in 15 experimental diets (Units/kg)…………………………………………………73

Table 2.5 Effects of dietary treatments on performance variables of birds fed Corn 1diets…………………………………………………………………………..74

Table 2.6 Effects of dietary treatments on performance variables of birds fed Corn 2 diets………………………………………………………………………….75

Table 2.7 Effects of dietary treatments on performance variables of birds fed Corn 3 diets………………………………………………………………………….76

Table 2.8 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 1 diets (DM basis)……………………………………………..77

Table 2.9 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 2 diets (DM basis)……………………………………………..78

Table 2.10 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 3 diets (DM basis)……………………………………………..79

Table 3.1 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 1 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis..……………………………………98

Table 3.2 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 1 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis..……………………………………99

Table 3.3 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 2 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis……………………………………100

Table 3.4 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 2 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis……………………………………101

Table 3.5 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 3 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis……………………………………102

Table 3.6 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 3 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis……………………………………103

Table 4.1 Composition of diets fed to broiler chicks…………………………..132

Table 4.2 Chemical composition of 8 test samples (%)-DM basis…………….133

Table 4.3 Chemical composition of 8 experimental diets (%)-DM basis………134

Table 4.4 Growth performance variables of birds during day 8 to 13……………135

Table 4.5 Apparent ileal and total tract digestibility coefficient of nutrients and energy of experimental diets (DM basis)……………………………………….136

Table 4.6 Relationships of test sample in vivo AME (chick bioassay) with physico-chemical characteristics and growth performance variables…………..137

Table 4.7 Regression equations for prediction of in vivo AME based on in vitro AME and other chemical characteristics of 8 test samples (kcal/kg of DM)…..138

Table 4.8 Prediction of in vivo AME value of test samples based on in vitro AME and 4 chemical characteristics (kcal/kg of DM)……………………………..139

Table 5.1 Composition of the experimental diets fed to broiler chicks ………………………………………………………………………………..160

Table 5.2 Analyzed physical characteristics and chemical composition of test samples (%)-DM basis………………………………………………………161

Table 5.3 Chemical analyses of experimental diets (%)-DM basis……………..162

Table 5.4 Enzyme activities in experimental diets (units/kg feed)…………….163

Table 5.5 Growth performance of broiler chicks fed experimental diets (day 8 to 14)…………………………………………………………………………164

Table 5.6 Apparent ileal and total tract digestibility of energy of experimental diets (%)-DM basis…………………………………………………………..165

Table 5.7 Effects of enzyme supplementation on AME of test samples determined in the bioassay (kcal/kg)-DM basis……………………………………………..…166

Table 5.8 Relationships of *in vivo* AME (with and without enzyme) with physico-chemical characteristics and growth performance variables……………………167

Table 5.9 Regression equations for prediction of in vivo AME (without enzyme) based on in vitro AME and other chemical characteristics of 8 test samples (kcal/kg of DM)…………….…………………………………….………….168

Table 5.10 Regression equations for prediction of in vivo AME (with enzyme) based on in vitro AME and other chemical characteristics of 8 test samples (kcal/kg of DM)…….…………………………………………….………….169

Table 6.1 Composition of diets fed to broiler chicks…………………………..195

Table 6.2 Physico-chemical characteristics of field pea samples (%) - DM basis……………………………………………………………………..196

Table 6.3 Analyzed amino acid content of field pea samples (%)-DM basis.....……………………………………………………………………197

Table 6.4 Physico-chemical characteristics of barley samples (%)-DM basis..……………………………………………………………..…..198

Table 6.5 Analyzed essential and non-essential amino acid contents of barley samples (%) - DM basis……………………………………………………...199

Table 6.6 Chemical analyses of field pea-based experimental diets (%)-DM basis……………………………………………………………………..200

Table 6.7 Chemical analyses of barley- based experimental diets (%)-DM basis……………………………………………………………………..201

Table 6.8 Growth performance variables of broiler chicks fed field peas-based experimental diets…………………………………………………………202

Table 6.9 Growth performance variables of broiler chicks fed barley-based experimental diets…………………………………………………………203

Table 6.10 Apparent ileal digestibility of nutrients and energy of field pea-based diets (%)-DM basis……………..……………………………………………204

Table 6.11 Apparent total tract digestibility of nutrient and energy of field pea-based diets-DM% basis………………………………………………………205

Table 6.12 Apparent ileal digestibility of nutrients and energy of barley-based diets (%) - DM basis……………………………………………………………206

Table 6.13 Apparent total tract digestibility of nutrient and energy of barley-based diets (%)-DM basis…………………………………………………………..207

Table 6.14 Relationships of in vivo AME of field pea and barely samples with physico-chemical characteristics and growth performance variables 208

List of Abbreviations

AA	Amino acid
ADF	Acid detergent fiber
AIA	Acid insoluble ash
AME	Apparent metabolizable energy
AMEn	Nitrogen-corrected apparent metabolizable energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BW	Body weight
CCAC	Canadian Council on Animal Care
CP	Crude protein
CPSR	Canada Prairie Spring Red
CV	Coefficient of variation
CWAD	Canada Western Amber Durum
CWHWS	Canada Western Hard White Spring
CWRS	Canada Western Red Spring
CWRW	Canada Western Red Winter
CWSWS	Canada Western Soft White Spring
DE	Digestible energy
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
FI	Feed intake
GE	Gross energy
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IDE	Ileal digestible energy
NaOH	Sodium hydroxide
NC	Negative control
NDF	Neutral detergent fiber
NIRS	Near infrared reflectance spectroscopy
NSP	Non-starch polysaccharides
PC	Positive control
PDIFF	Probability of difference
r	Correlation coefficient

R^2	Coefficient of determination
RSD	Residual standard deviation
SEE	Standard error of estimate
SEP	Standard error of prediction
SNK	Student-Newman-Keuls
TI	Trypsin inhibitor

Chapter 1

Review of literature[1]

1.1 Introduction

Feed is the major portion of the variable costs in intensive poultry production systems around the world (Leeson, 2004; Zijlstra and Beltranena, 2007). It is estimated that this expenditure can be up to 75% of the total cost of production in commercial poultry operations. However, this percentage may fluctuate due to many variables dictated by regional or international situations including instability in commodity prices. For instance, instability in feed prices has resulted from the rapid expansion of the biofuel industry, which has been accompanied by the removal of substantial quantities of cereal grains from the global animal feed industry (Animal Nutrition Association of Canada, 2009; Best, 2009; Patience et al., 2009).

Wheat is a common cereal feedstuff for commercial poultry rations and it generally has lower energetic value than corn, although its protein content is higher (Wiseman, 2000; Leeson and Summers, 2005; Coon, 2005; Carre et al., 2007). There are several hundreds varieties of wheat being used for human food and animal feed in different parts of the world (Carre et al., 2007). The annual global production of wheat is more than 600 million tonnes. The European Union,

[1]A version of this chapter has been published (Yegani, M and D. R. Korver. Prediction of variation in energetic value of wheat for poultry. 2012. Can. J. Anim. Sci. 92:261-273).

U.S.A, Canada, Australia, and Argentina are the major producers of wheat (McFall and Fowler, 2009), accounting for 138.82, 60.37, 26.85, 21.92, and 11.00 million tonnes, respectively for 2009/2010 (United States Department of Agriculture, 2011).

1.2 Variations in energy availability in wheat

Variations in physico-chemical characteristics of wheat can significantly influence nutrient availability and growth performance of the animals. This is a concern that must be considered when formulating diets (Svihus and Gullord, 2002; Scott et al., 2003; Opapeju et al., 2007; Gutierrez del Alamo et al., 2008a). As shown in Table 1.1, a wide range in apparent metabolizable energy (**AME**) values of wheat samples is reported in different studies. As previously reviewed (Hughes and Choct, 1999; van Barneveld, 1999; Gutierrez-Alamo et al., 2008b), there are a large number of factors that can, to different extents, contribute to variability in the physico-chemical properties of feedstuffs. In the following section of this chapter, some of these contributing factors are briefly discussed. However, practical approaches that can be used to predict these variations are presented later in this chapter.

As shown in Table 1.2, wheat cultivar, environment-related factors, and storage conditions can all affect AME of wheat for poultry through influencing the nutrient profile of wheat (e.g., fiber content, concentrations of anti-nutritional factors, and starch content) which may then be reflected as variations in nutritive value for birds (Svihus and Gullord, 2002).

1.2.1 Wheat cultivar

Scott et al. (1998b) evaluated 108 wheat samples of 9 cultivars (collected from different locations in two consecutive years) and observed that cultivar is one of the factors that play an important role with respect to feeding value of wheat samples.

Although there are different categories of wheat, classification into soft and hard wheat is of practical relevance when it comes to assessing the nutritional quality of wheat (Leeson and Summers, 2005). Peron et al. (2006) observed that ileal digestibility of starch in 3-wk-old broiler chickens fed with a soft wheat was 6% higher than in birds fed a hard wheat cultivar. In a follow-up study, it was shown that starch granules were trapped within coarse particles in the hard wheat and the number of coarse particles was higher in the ileal digesta of birds fed this wheat (Peron et al., 2007). Microscopic evaluation of the ileal digesta showed that the amount of undigested starch granules within the coarse particles of the digesta of birds fed hard wheat diets was significantly higher than that of the birds fed soft wheat diets. It was suggested that the presence of higher levels of coarse particles in hard wheat may reduce accessibility of starch-digesting enzymes to the substrates (Peron et al., 2007). This situation may have resulted from the strong association between starch and protein in hard wheats (Leeson and Summers, 2005).

The physical structure of wheat (i.e., hardness or softness) affects both the outcome of processing techniques (e.g., a positive correlation exists between hardness and pellet durability) in wheat-based diets and also nutrient content (e.g.,

hard wheats tend to have higher starch-bound protein). Also, starch digestibility tends to be negatively correlated with the hardness of wheat samples (Carre et al., 2005; Leeson and Summers, 2005).

1.2.2 Environment-related factors

The effects of environmental conditions on the nutritional quality of wheat can be as important as the effects of cultivar-associated factors (Anderson and Bell, 1983). Kim et al. (2003) reported a negative relationship between rainfall and bushel weight and CP, but rainfall was positively correlated with the total starch and soluble non-starch polysaccharides (**NSP**) concentrations. There were also significant negative relationships ($P < 0.01$) between rainfall and other components such as acid detergent fiber (**ADF**) and lignin in wheat samples.

Environmental conditions can affect NSP content of wheat samples, resulting in variations in AME value of wheat. Choct et al. (1999a) observed a considerable variability in AME values of wheat samples collected during 3 harvest seasons. Dry and hot weather conditions during the growth period of wheat elevated the NSP concentrations, resulting in reduced AME values of the grain. In the study of Coles et al. (1997), a positive relationship was found between NSP (arabinoxylan) content of wheat samples and drought, and a negative relationship between arabinoxylan content and AME values of these samples.

1.2.3 Anti-nutritional factors

Although a wide range of anti-nutritional factors are found in wheat, NSP are among the most extensively studied (Iji, 1999; Leeson and Summers, 2001).

NSP are generally divided into 2 groups: water soluble and insoluble (Guenter, 1993). Although both groups can have anti-nutritional activities, soluble NSP can be more problematic in wheat-based poultry diets, resulting in the presence of viscous materials in the intestinal lumen (Annison, 1991; Guenter, 1993; Choct et al., 1995; Choct, 2006). In wheat, arabinoxylans constitute the major portion of the NSP profile (Annison, 1991; Austin et al., 1999; Zijlstra et al., 1999; Steenfeldt, 2001).

Soluble NSP decrease the digestion and absorption of nutrients due to increased intestinal digesta viscosity (Choct et al., 1996; Choct et al., 1999b). The presence of undigested and unabsorbed nutrients can also increase bacterial fermentation in the small intestine. High viscosity of the gut contents may inhibit efficient exposure of starch granules to amylase. This exposure is a necessary step for enzymatic digestion of starch in the small intestine. If this step does not occur, undigested starch is fermented by bacteria, leading to increased concentrations of volatile fatty acids in the ileum. Increased fermentation can have negative impacts on production performance of the birds (Choct et al., 1996).

1.2.3.1 NSP and AME values

As previously noted, AME values can vary among different wheat samples (Annison, 1991; Choct et al., 1999a; Rafuse et al., 2005). There is generally a negative relationship between NSP content of wheat samples and their AME values (Annison, 1991; Austin et al., 1999; Choct et al., 1999a; McCracken et al., 2008). Choct et al. (1995) reported that the soluble NSP content of a low AME wheat was higher than that of normal wheat (19.0 compared to 13.2 g/kg,

respectively). AME values of low and normal wheats were 12.02 and 14.52 MJ/kg, respectively. A similar pattern was also observed for digesta samples, as soluble NSP content of digesta was greater in birds fed with the low AME wheat compared to birds that had received normal wheat-based diets. In another study, Coles et al. (1997) reported that there was a negative correlation between starch level and arabinoxylan concentration in wheat. These observations might, to some extent, explain the negative impact that high levels of arabinoxylans can have on AME value of wheat for poultry.

1.2.4 Starch content

Starch is the main energy-providing polysaccharide in cereal grains including wheat, although its concentrations can vary within and among feedstuffs (Bach Knudsen 1997; Wiseman, 2006). As reviewed by Carre (2004), a number of factors including the structure of starch granules (e.g., amylose content of granules), anti-nutrient compounds (e.g., concentrations of α-amylase inhibitors and soluble NSP), and accessibility to starch granules (wheat hardness and its negative relationship with starch digestibility) may contribute to variations in digestibility of starch in feedstuffs including wheat, which can subsequently affect the energetic value of the diets for poultry.

According to Wiseman (2006), starch digestibility and AME values of wheat are correlated and variability in concentration and digestibility of starch can influence AME of wheat or wheat-based diets. Svihus and Gullord (2002) reported a positive relationship between starch content and AME value of wheat diets in broiler chickens. Considering that starch provides a substantial portion of

energy of wheat, low starch digestibility can therefore significantly contribute to low AME values of wheat-based diets. In that study, there was also a negative correlation between protein content and AME values. It was suggested that the protein matrix could have possibly impeded the availability of starch in the endosperm of wheat samples.

1.2.5 Wheat storage

"New crop syndrome" refers to negative consequences that feeding of newly harvested grains such as wheat may have on production performance of broiler chickens (Scott and Pierce, 2001). Extended storage times can influence nutritive value of wheat including changes in the AME levels. However, it must be noted that these effects are dependent on wheat cultivar as well as length and conditions of storage (Choct et al., 1995; Scott and Pierce, 2001; Kim et al., 2003; Pirgozliev et al., 2006).

The AME values of wheat samples for poultry increased substantially from 9.18 to 12.02 MJ/kg as a result of storage for 1 year. Activity of wheat endogenous enzymes (e.g., glycanases) during storage may enhance the nutritional values of low AME wheat during prolonged storage (Choct et al., 1995). Kim et al. (2003) reported that storage of wheat for 6 months resulted in significant decreases in soluble NSP, ADF, and lignin contents. The endogenous enzymes present in wheat may have broken down complex molecules of NSP into smaller molecules such as free sugars.

In another study, Jood et al. (1993) reported a significant increase in the concentration of sugars (i.e., total soluble sugars, reducing and non-reducing

sugars) in cereal grains including wheat as a result of storage for 4 months. This increase likely resulted from starch degradation during storage, as there was a 9 to 14% reduction in the starch content of these grain samples. Pirgozliev et al. (2006) also found that starch hydrolysis can occur during storage due to the activity of endogenous enzymes, resulting in the elevation of free sugar levels in stored wheat.

1.2.6 Animal-related factors

In addition to ingredient-related factors, bird-dependent parameters (e.g., age, sex, strain, and health condition) can also contribute to variations in nutrient availability of feedstuffs within and among birds (Scott, 1996; van der Klis, 2010). However, only differences in the GI tract of birds are briefly discussed here. Maisonnier et al. (2001) observed that variations in digestibility of nutrients and nitrogen-corrected AME **(AMEn)** in broiler chickens can be, to some extent, caused by variations in characteristics of different segments of the GI tract such as weight: length ratio of duodenum and jejunum. The digestibility coefficients of nutrients were highest in birds that had the greatest weight: length ratio of duodenum and jejunum. For instance, there was a significant positive correlation between duodenum weight:length ratio and AMEn of the diet. De Verdal et al. (2010) reported that in addition to differences in weight and length of different sections of the GI tract, there were also histological differences in the small intestine between two different genetic lines of broiler chickens. These variations might have played a role in differences in AMEn value of wheat-based diets (3,278 kcal/kg vs 2,455 kcal/kg, respectively) in these birds. For example, birds

with lower dietary AMEn had greater villus height (by 14 to 16%) and crypt depth (by 10 to 15%), higher number of goblet cells (by 27 to 34%), and thicker muscle layers (by 17 to 24%) compared to birds with higher dietary AMEn (De Verdal et al., 2010).

1.3 Prediction of variations in nutrient availability of wheat

Variations in nutritive values of wheat can result in inefficiencies in diet formulation, particularly in terms of energy and amino acids. The inefficiencies (i.e., over- or under-formulations) can have negative impact on production performance of birds as well as the environment, which ultimately results in reduced profit of the producers (Scott, 1996; van Kempen and Simmins, 1997; Patience et al., 2009). Thus, nutritionists need to not only have knowledge of the nutritional requirements of commercial poultry, but they must also be able to determine or predict nutritive value of each batch of feedstuffs in an accurate and timely manner (van Kempen and Simmins, 1997).

Attempts have been made to predict nutritive value of feed ingredients or finished diets through five main methods: prediction by using physical characteristics; chemical analyses; prediction by in vivo digestibility (i.e., animal trials or bioassays); prediction by using in vitro techniques; and prediction by using near infrared reflectance spectroscopy (**NIRS**) technology (Carre, 1991; Leeson, 1997; van Kempen and Simmins, 1997; Hughes and Choct, 1999; Losada et al., 2009; 2010). Physical measurements are not a good predictor of nutrient content, and although chemical analyses and animal trials remain important aspects in prediction systems, their use is limited under practical situations. In

vitro digestibility and NIRS can, to some extent, fill the gap when it comes to rapid evaluation of wheat nutritional quality. The main focus of the following section of this chapter will be on in vitro digestibility assays and NIRS. With supporting data generated by chemical analysis techniques and in vivo feeding trials, in vitro digestibility methods can be used to generate accurate databases for NIRS, which in turn can be used to predict, in a "real-time" approach, feeding value of wheat for poultry.

1.3.1 Physical characteristics

Measuring physical characteristics of grains (e.g., bushel weight and 1,000 kernel weight) is normally a faster and less expensive approach as compared to chemical analyses (Lilburn and Dale, 1989; Fairbairn et al., 1999). However, physical characteristics should not be considered separately from other characteristics when it comes to nutritional quality evaluation because many factors other than physical characteristics can also play a role in this regard (Dale, 1994). There are several published studies which have investigated the relationship between physical characteristics and nutritive value of wheat (Garnsworthy et al., 2000; McCracken et al., 1999; 2008; McCracken and Quintin, 2000; Wiseman, 2000). However, according to these studies, physical characteristics are not generally considered as good predictors of nutritional quality for poultry. Wiseman (2000) reported no significant correlations between bushel weight and 1,000 grain weight with the AME values of 50 wheat samples (from 10 varieties) fed to broiler chickens.

1.3.2 Chemical analyses

Proximate analyses have been extensively used for many years to measure nutrient content of feedstuffs and diets (Carre, 1991; Noblet and Perez, 1993; Fairbairn et al., 1999; Zijlstra et al., 1999; Zijlstra, 2006). However, chemical analyses are not the best choice for decision making when it comes to feed quality assessment at a commercial level, because of the limitations associated with this approach. Reproducibility of the chemical measurements, time required for the analyses, cost, need for specific equipment for laboratory procedures, and production of waste materials are all amongst the most common limiting factors (Leeson, 1997; Fairbairn et al., 1999; Zijlstra, 2006; Noblet and Jaguelin-Peyraud, 2007; Losada et.al., 2009; 2010). More importantly, chemical analyses only provide information on the total nutrient content of feed ingredients or diets without looking into the digestibility of a given feedstuff or diet in the animal. As noted previously, digestibility of wheat or diets can be significantly affected by a wide variety of factors and chemical analyses are not able to take all these factors into consideration (van Barneveld, 1999; Boisen, 2000).

1.3.3 In vivo digestibility

Results of animal digestibility trials or bioassays are the most accurate way to determine actual nutrient digestibility because ultimately, it is the animal response to variation in diet AME that is of utmost importance to the industry. However, animal trials are time-demanding, costly in terms of both personnel and facilities, require large amount of samples (i.e., ingredients or finished feed), surgical interventions are necessary in some instances, and more recently, there

are growing welfare-related issues regarding the use of animals in digestibility trials. These limitations have made bioassays a less desirable choice for routine feed quality evaluation (Furuya et al., 1979; Boisen and Eggum, 1991; Carre, 1991; Fuller, 1991; McNab, 1991; Leeson, 1997; Scott et al., 1998a; Huang et al., 2000; Weurding et al., 2001; Zijlstra, 2006; Losada et al., 2009; 2010).

1.3.4 In vitro digestibility

Due to limitations associated with the above-mentioned predictive approaches, there is an increasing interest in using rapid methods such as in vitro digestibility techniques as part of feed quality evaluation programs (Graham, 1991; Fuller, 1991; McNab, 1991; Boisen, 2000; Zijlstra, 2006). Due to the complexity of digestive processes in the GI tract of the animals, every effort should be made to simulate these processes as closely as possible in the in vitro analyses (Boisen and Eggum, 1991; Longland, 1991; Huang et al., 2000). Information on in vitro digestibility techniques in poultry is limited. However, much more work has been done in swine, and approaches developed in that regard may be beneficial to an understanding of in vitro digestibility studies in poultry.

A good in vitro digestibility technique should be simple, rapid, accurate, and be able to generate reproducible predictions of in vivo responses (Furuya et al., 1979; Fuller, 1991; Graham, 1991; Boisen 2000; Huang et al., 2000; Noblet and Jaguelin-Peyraud, 2007; Regmi et al., 2008; 2009). Any in vitro digestibility technique must be validated and this validation should be done by comparing the in vitro values with the corresponding data collected from animal studies using the same ingredients or diets. The higher the correlation between the in vitro and

in vivo results, the greater the strength of the predictive values (Sakamoto et al., 1980; Boisen and Eggum, 1991; Graham, 1991; Boisen and Fernandez, 1995; Boisen, 2000).

One of the in vitro techniques that have been extensively studied over the years is the “filtration” method (Boisen and Eggum, 1991). The filtration method is designed to predict ileal digestibility of protein and energy and also total tract digestibility of energy. In this method, 2 (simulation of stomach and small intestine) or 3 (simulation of the total tract) successive incubation steps are used (Boisen and Eggum, 1991; Boisen, 2000). In the two-step method, pepsin and pancreatin (a mixture of amylase, lipase, and protease) are used, whereas in the three-step approach, in addition to pepsin and pancreatin, a mixture of other enzymes (e.g., cellulase, hemicellulase, xylanase, and β-glucanase) is also used in order to mimic fiber degradation in the ceca. It is important that the in vitro pH values are maintained (by adding different buffers into the in vitro flasks) within ranges that mimic as closely as possible the specific regions of the digestive tract (Boisen and Eggum, 1991; Boisen, 2000; Regmi et al., 2009). For pepsin (mimicking stomach digestion) and pancreatin (simulating small intestinal digestion) incubation phases of the in vitro digestion, pH values should be about 2 and 6.8, respectively (Clunies and Lesson, 1984; Boisen and Eggum, 1991; Boisen, 2000; Losada et al., 2009; 2010). The incubation temperature should also be as close as possible to chicken body temperature (41°C) throughout the whole in vitro digestion process (Bennett et al., 1986; Annett et al., 2002).

At the end of the final incubation step, flasks are removed from the shaking water bath and content of flasks are filtered. The filtrate (i.e., undigested residue) is then used for different measurements such as DM, GE, and nitrogen. The difference between the nutrient content of the original sample and the in vitro residue of that sample is the in vitro digestibility value. The assumption for in vivo digestibility trials is that nutrients and energy that disappear from the digestive tract have been absorbed by the animal; the filtration step mimics the absorption process in vitro. These values are then related to the corresponding in vivo digestibility coefficients in order to develop prediction equations. If the prediction accuracy is high, then the in vitro technique can be used to predict the in vivo digestibility of a given feed ingredient or diet in the animal (Boisen and Eggum, 1991; Boisen, 2000; Regmi et al., 2009).

1.3.4.1 Important considerations for developing an in vitro digestibility technique

1.3.4.1.1 Physical characteristics of samples

Sample weight, particle size, and physical form of the samples can affect the accuracy of results produced by the in vitro methods. Although this may vary depending on the type of ingredient, it is suggested that sample weight should be 0.5 g (Boisen and Eggum, 1991; Boisen and Fernandez, 1997), because a sample size of 1g appears to result in underestimation of the in vitro digestibility coefficients.

The particle size is another important factor which influences the extent of enzyme access to nutrients through increases in the surface area. In the GI tract of the birds, particle size reduction is generally achieved by the action of the gizzard

(Clunies and Leeson, 1984). In order to mimic the gizzard physiological functions in the in vitro system (Weurding et al., 2001), the particle size of the samples for the in vitro analyses should always be smaller (e.g., 1 mm or smaller) than used in the in vivo digestibility trials which is usually about 2-3 mm (Boisen and Eggum, 1991; Boisen and Fernandez, 1997; Weurding et al., 2001; Huang et al., 2003; Regmi et al., 2008; 2009; Losada et al., 2010). Smaller particles in the in vitro environment provide a larger surface area per unit of mass, which results in faster access of enzymes to the nutrients, leading to increased accuracy of prediction of the in vivo digestibility (Clunies and Lesson, 1984). This is likely due to the particle size reduction of feed in the gizzard of the bird, which is absent in the in vitro model.

A series of studies (Parsons, 1991; Parsons et al., 1991) showed that there should be a consistency in the particle size within the same type of samples used in the in vitro digestibility assay as this factor can affect repeatability of the results. In another study on 15 different feedstuffs (e.g., protein sources and cereal grains), Furuya (1991) reported that the in vitro digestibility of CP in samples with the particle size of 0.5 mm was higher than for samples of 1 mm, although this difference varied based on the type of ingredient tested. For instance, the in vitro digestibility of protein of corn samples with the particle size of 0.5 and 1 mm was 83 and 73%, respectively, whereas, these values for wheat were 91 and 90%.

The physical form of samples is another important factor in the in vitro methodology. Noblet and Jaguelin-Peyraud (2007) reported that the in vitro

prediction equations generated from mash diets may not provide an accurate estimate of the in vivo digestibility of energy of pelleted diets in pigs. Thus, it is necessary to use different regression equations to predict the in vivo digestibility of mash and pelleted diets. More studies are also required to investigate the effects that feed processing techniques may have on the results of the in vitro digestibility.

1.3.4.1.2 In vitro digestion enzymes

Enzyme-related parameters (e.g., type, concentrations, and also enzyme-substrate specificity) can also affect the in vitro digestibility results. Regmi et al. (2009) reported that a greater amount of pepsin (25 vs. 10 mg/ml) and pancreatin (50 vs 100 mg/ml) and also addition of Viscozyme (a mixture of different fiber-digesting enzymes) increased the accuracy of prediction of the in vivo digestibility of energy of 20 wheat samples in grower pigs. Boisen and Fernandez (1997) showed that the individual enzymes used in each incubation step can have different effects on digestibility of OM in various feed ingredients. Pepsin had a more pronounced effect on soybean meal (50% protein content) compared to barley (approximately 12% protein). On the other hand, pancreatin had a greater impact on barley than soybean meal due to the high starch concentration in barley.

One of the important limiting factors regarding the activity of enzymes is the pH of the in vitro digestion system. The pH of the incubate in each of the in vitro digestibility stages should mimic, as closely as possible, the pH of different regions of the digestive tract of the animals (Clunies and Lesson, 1984; Ao et al., 2008). In the study of Clunies and Lesson (1984), there were no differences in the

in vitro digestibility of DM at pH of 6.6 to 6.9. However, there was a significant reduction in the digestibility at pH of 6.5. It was suggested that pancreatic α-amylase, as the main contributing factor to the digestibility of DM in the intestinal phase, probably continued to function in the pH range of 6.6 to 6.9 and as a result, there was no reduction in the in vitro digestibility. It is therefore important to monitor and adjust the pH values of the incubate during the in vitro digestion phases as various ingredients or diets may have different buffering capacity (Clunies and Lesson, 1984).

1.3.4.1.3 Continuous mixing

It is important that the contents of the in vitro flasks are continually mixed during the whole incubation process to ensure that the nutrients are efficiently degraded due to constant contact with enzymes (Boisen and Eggum, 1991; Longland, 1991; Boisen and Fernandez, 1997). Ideally, the end-products of the in vitro digestive processes should be constantly removed from the in vitro environment, because presence of these end-products may negatively influence the enzymatic activity. This removal does not occur in the filtration method as opposed to the digestive tract of the animals, but it seems that this is not of significant importance considering that there is generally a “surplus” of enzymatic activity in the in vitro environment. There are more complex in vitro methods in which digestion products can be removed, but these techniques may, due to their complexity in implementation including specific requirements for equipment, not be practical when it comes to routine feed quality evaluation (Boisen and Eggum, 1991; Longland, 1991; Boisen, 2000). Another consideration is related to the roles

that gut microflora may play in nutrient digestion in the digestive tract of the animal (Boisen and Eggum, 1991). These functions might not be necessarily simulated in the in vitro digestibility techniques.

1.3.4.2 Accuracy of in vitro methods

It is necessary to determine the accuracy and precision of prediction equations of an in vitro method relative to in vivo results using statistical analysis (i.e., regression) which can provide information such as coefficient of determination (R^2), residual standard deviation (**RSD**), and standard error of prediction (**SEP**) values. These values are considered as measures of the quality (i.e., accuracy and precision) of prediction of the in vivo digestibility from the in vitro results. Obtaining high R^2 and low RSD or SEP values indicates that the prediction equations are reliable (Furuya et al., 1979; Clunies et al., 1984; Furuya, 1991; SAS Institute, 2002; Regmi et al., 2008; 2009).

In vitro digestibility values are usually expected to be greater than the in vivo data of the same samples. One of the main reasons for this difference could be related to minimal or lack of endogenous losses of nutrients in the in vitro digestibility methods (Furuya et al., 1979; Boisen and Eggum, 1991; Boisen and Fernandez, 1995; Boisen, 2000; Huang et al., 2003; Regmi et al., 2008; Wilfart et al., 2008). As reported by Boisen and Fernandez (1995), it must be noted that endogenous losses generally do not occur to a similar extent for all ingredients in the in vivo digestibility assays. In other words, endogenous losses of nutrients can vary substantially based on chemical characteristics of ingredients or finished diets.

1.3.4.3 Use of in vitro digestibility methods

Although the available information on in vitro digestibility techniques for prediction of energetic values of ingredients or complete diets in poultry is limited (Table 1.3), these techniques can be an efficient and practical approach to develop specific regression equations for prediction of ileal or total tract digestibility of nutrients and energy (Clunies and Leeson; 1984; Clunies et al., 1984; Valdes and Leeson, 1992b; Losada et al., 2009; 2010). However, these studies have some limitations which will be discussed later.

In the study of Losada et al. (2009), 94 batches of different cereals including wheat and their by-products were tested for the in vitro digestibility of DM and OM to predict the energetic value of these ingredients for poultry. The R^2 between the in vitro digestibility of DM and OM with the in vivo AMEn were 0.59 and 0.62, respectively. These R^2 values are not considered to be high, and therefore the predictions generated are not likely to reflect actual animal response. Valdes and Leeson (1992b) used a two-step incubation method (pepsin treatment followed by pancreatin, bile salts, and enterokinase treatment) to predict the AMEn of 71 diets for poultry. This method was able to provide a reasonable prediction of AMEn (R^2= 0.71). The difference between the in vitro digestible energy and the in vivo AMEn values in 30 diets (out of 71 diets) was below 100 kcal/kg. However, this difference for the other 41 diets ranged from 100 to more than 400 kcal/kg. The authors concluded that this method cannot be recommended as a routine procedure for prediction of AMEn in poultry diets on a global basis, because the difference between the in vitro digestible energy and the in vivo

AMEn was not acceptable for most of the diets. Another explanation by these researchers was that poultry diets are composed of various ingredients (at different inclusion rates) and this can result in complications in terms of providing the in vitro technique requirements such as pH, enzyme levels, and duration of incubation phases (Valdes and Leeson, 1992b). For instance, poultry diets generally vary in their buffering characteristics and due to this, the optimum pH might not be attained in the in vitro system for all diets (Clunies and Lesson, 1984; Valdes and Leeson, 1992b). Based on the above-mentioned requirements, Valdes and Leeson (1992b) suggested that specific in vitro digestibility techniques should be developed for each feedstuff used in poultry diets. In addition, according to Zijlstra et al. (2010), specific in vitro digestibility methods are required for each feed ingredient in order to predict the in vivo digestibility of nutrients and energy in pigs.

Although previously described in vitro digestibility techniques for poultry have provided valuable information (Clunies et al., 1984; Valdes and Leeson, 1992b; Losada et al., 2009; 2010), they were mostly validated with the in vivo data determined in adult roosters. This is a limiting factor that should be taken into consideration in the application of these in vitro digestion assays results (van der Klis, 2010). Svihus and Gullord (2002) reported that determination of AME of diets in adult roosters resulted in significantly higher values compared to determination in broiler chicks. Thus, these AME values may not necessarily reflect the actual energetic value of a specific feedstuff or diet for broiler chickens. In that study, AME values of wheat-based diets in broiler chicks and

adults roosters were 11.1 to 13.3 and 14.4 to 15.8 MJ/kg, respectively. As a result, it is important that in vitro data intended for broiler chicks are validated with the in vivo data from broilers and not roosters.

1.3.4.4 Application of in vitro digestibility results

An important reason for the continued development of accurate in vitro digestibility assays is the ability to generate a large database of digestibility results. These data are essential for the creation and expansion of calibration databases for NIRS technology in order to rapidly and accurately predict nutritional values of feed ingredients. This can provide an excellent opportunity for formulation of more balanced commercial rations for the animals (Boisen and Eggum, 1991; Graham, 1991; Regmi et al., 2008; 2009).

1.3.5 Near infrared reflectance spectroscopy (NIRS)

1.3.5.1 Basic concept

Animal and plant tissue is composed largely of hydrogen-containing bonds (e.g. C-H, N-H, and O-H). The types and quantity of these bonds in each tissue are normally determined by the chemical nature of that specific tissue. The NIRS technique measures the absorption of light energy by these bonds (within a sample) at specific wavelengths in the near infrared region. When a sample is irradiated by near infrared light (wavelength of 750-2,500 nm), some portions of the light is absorbed by these bonds and the reflected light, which is an indirect indication of the absorbed light, provides information on the chemical characteristics of that specific sample. This spectrum (the reflected or absorbed light) is then related to samples of known content (reference values) by applying

statistical models to develop calibration equations. These equations are then used to estimate the nutrient profile of unknown samples (Leeson, 1997; Foley et al., 1998). It is of critical importance that reference values used for calibrations represent a wide range in values of the characteristic or measurement of interest so that robust calibrations can be developed (Zijlstra et al., 2011).

1.3.5.2 NIRS applications

The NIRS has gone through several phases of development over the past 40 years. At the beginning, this technology was used to predict concentrations of components such as protein and moisture in ground samples. Subsequently, it was applied to other components such as fiber as well. Later on, this technology was also used to analyze nutrient composition of whole grains and seeds (Williams and Norris, 2001a).

Available evidence indicates that there has been an increasing trend in the use of this technology in the agriculture, food, pharmaceutical, medicine, and environmental-related areas (Givens et al., 1997; Leeson, 1997; Foley et al., 1998; Pires et al., 2001; Rose et al., 2001; Smith et al. 2001; Williams and Norris, 2001a; Kays and Barton, 2002; 2004; Xing et al., 2008).

The NIRS technology has the potential to provide a fast and economically feasible predictive tool to help formulate rations as accurately as possible in order to achieve the desired growth performance in animals. This technology has been shown capable of predicting digestible nutrient content of feed ingredients faster in comparison to traditional feed quality evaluation methods (Aufrere et al., 1996;

van Kempen and Simmins, 1997; van Kempen and Bodin, 1998; Garnsworthy et al., 2000; Fontaine et al., 2002; Losada et al., 2009; 2010; Owens et al., 2009).

The NIRS technology is non-destructive, requires little sample preparation and chemical substances, and as a result, no chemical waste is produced (Valdes and Leeson, 1994; Aufrere et al., 1996; Leeson, 1997; Foley et al., 1998; Kays and Barton, 2004). It generally takes about 2-5 minutes to obtain the results from NIRS and this information can be used to reduce or minimize nutrient imbalances in commercial rations fed to the animals. Formulating balanced diets also reduces the rate of excretion of nutrients (through manure) into the environment (Scott, 1996; van Kempen and Simmins, 1997; Pujol et al., 2007).

1.3.5.3 NIRS limitations

There are potential errors associated with NIRS technology. Sources of errors are classified into three main groups: sample-related errors, reference method (e.g., wet chemistry)-dependent errors, and NIRS method errors (Valdes et al., 1985; Foley et al., 1998; Hruschka, 2001; Williams and Norris, 2001b). Factors associated with samples (e.g., particle size) or sampling (e.g., non-representative samples) are generally the main contributing elements to differences between reference method values and NIRS results (Hruschka, 2001; Williams and Norris, 2001b). The quality of NIRS predictions is dependent on the accuracy and repeatability of reference method used for the calibrations (Foley et al., 1998). This underscores the importance of highly repeatable and accurate in vitro digestibility methods.

1.4 The future of feed quality evaluation

Although costs associated with establishing an NIRS system (cost of the machine itself and ongoing calibrations) is still relatively high (Patience et al., 2009), this technology will undoubtedly gain more popularity in the industry for routine feed quality assessments. The NIRS equipment will likely become more available in order to allow more flexibility in terms of real-time on-site applications for prediction of variations in nutrient availability. Considering that physical and chemical characteristics of feed ingredients constantly change, it is essential to regularly update the calibration databases in order to have accurate NIRS predictions (Givens et al., 1997; Leeson, 1997; Foley et al., 1998; Owens et al., 2009; Patience et al., 2009). Up-dating of calibration databases should be an on-going effort involving adding larger groups of samples (preferably from various geographical locations) in order to enhance the accuracy and also applicability of NIRS predictions to different parts of the world (Valdes and Leeson, 1992a, 1994; Leeson, 1997; van Kempen and Bodin, 1998; Fontaine et al., 2002; 2004; Pujol et al., 2007; Owens et al., 2009). Fontaine et al. (2002; 2004) reported that NIRS calibration equations can also be transferred between laboratories in order to facilitate estimation of nutritional quality of different feed ingredients at both national and international levels.

1.5 Conclusions

There are variations in AME content of wheat samples used in poultry feeding. It is important to have a good understanding of these variations as they are directly associated with meeting commercial bird's nutritional requirements.

Ideally, nutritionists will be able to know the level of nutrient bioavailability of individual feedstuffs at the time they formulate a diet. Physical characteristics of wheat are poorly related with feeding quality for poultry, and are therefore not suitable for real-time evaluation of wheat quality for poultry. Chemical analyses of wheat cannot be done in real-time, and animal trials are lengthy and expensive. In vitro digestibility techniques, incorporating chemical analyses, and validated with comparison of in vivo bird responses can be developed to provide the large amount of data necessary for robust NIRS predictions of feeding values. Once validated, and with ongoing database maintenance, NIRS can be used to predict ingredient feeding value in real time. These practical approaches can provide an opportunity for more accurate formulation of diets in the poultry industry.

1.6 Hypotheses and objectives

Accurate estimates of the quantity of available nutrients in feedstuffs provide a strong basis to meet nutrient requirements of animals and reduce or minimize the rate of over- or under- formulation of the diets. However, a lack of adequate or up-dated information in this regard usually necessitates that margin of safety of nutrients should be increased at time of diet formulation to ensure that the animals receive established levels of nutrients which in turn can result in an increase in feed costs (Fairbairn et al., 1999; de Lange and Birkett, 2005; Patience et al., 2009).

It is of economic importance to accurately evaluate and also predict variations in nutritive value of feed ingredients such as wheat, barley, and field pea for western Canadian animal industries in particular and Canada, in general

(Feed Quality Evaluation/NIRS ACIDF Project, 2007). Corn is also, to a limited extent, used in poultry rations in western Canada especially when wheat prices are higher than imported corn and this substitution can be accompanied by improved economic benefits to the poultry producers (Korver and Zuidhof, 2004).

The hypotheses of the present PhD thesis were: Nutrient availability of feed ingredients can vary substantially for broiler chicks; exogenous enzymes can reduce these variations; in *vitro* digestibility techniques can predict the variations. The objectives of the thesis, as part of a larger feed quality evaluation project in the province of Alberta (Feed Quality Evaluation/NIRS ACIDF Project, 2007), were as follows:

1- To evaluate variations in nutrient availability of corn, wheat, barley, and field pea samples through conducting *in vivo* digestibility trials in broiler chickens (Chapters 2, 3, 4, 5, 6).

2- To investigate the potential effects that exogenous enzyme supplementation may have on reducing variations in nutrient availability, enhancing nutrient digestibility and production performance of broiler chickens fed corn- or wheat-soy diets (Chapters 2, 3, 5).

3- To develop an *in vitro* digestibility technique to predict AME content of different wheat samples (varying in nutritive value) for broiler chickens (Chapter 4).

4- To investigate if the *in vitro* digestibility technique could also predict response of an NSP-degrading enzyme on AME value of wheat samples for broiler chickens (Chapter 5).

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Table 1.1 Variations in AME[1] values of wheat samples reported in different studies

AME (kcal/kg)	Number of samples tested	Method of AME determination	Reference
2,687 to 3,246	13	Total excreta collection-broiler chicks	Annison (1991)
1,992 to 3,282	12	Total excreta collection-broiler chicks	Austin et al. (1999)
3,250 to 3,660	54	Excreta-Celite as marker-broiler chicks	Bedford et al. (1998)
2,193 to 3,578	81	Total excreta collection-broiler chicks	Choct et al. (1999a)
1,839 to 3,320	160	Total excreta collection-broiler chicks	Garnsworthy et al. (2000)
3,363 to 3,590	30	Total excreta collection-broiler chicks	McCracken et al. (2008)
2,627 to 3,798	22	Total excreta collection-broiler chicks	Mollah et al. (1983)
3,026 to 3,533	94	Total excreta collection-broiler chicks	Owens et al. (2009)
3,012 to 3,344	8	Ileal digesta-Celite as marker-broiler chicks	Rafuse et al. (2005)
3,340 to 3,480	30	Excreta-Celite as marker-broiler chicks	Scott et al. (1998a)
3,280 to 3,650	108	Excreta-Celite as marker-broiler chicks	Scott et al. (1998b)
2,028 to 2,974	50	Total excreta collection-broiler chicks	Wiseman (2000)

[1]Apparent metabolizable energy

Table 1.2 Factors causing variations in AME[1] values of wheat samples

Factor	Possible mechanisms of action	Reference
Cultivar	Physical structure of wheat (i.e., soft vs. hard wheat) which can influence starch digestibility	Carre et al.(2005) Peron et al. (2006; 2007) Scott et al. (1998b)
Anti-nutritional factors	Negative relationship between NSP content and AME value of wheat samples	Annison (1991) Austin (1999) Choct et al. (1999a)
Environment	Weather conditions (e.g., dry/hot weather or drought) can increase NSP levels in wheat, negatively affecting AME value	Coles et al.(1997) Choct et al. (1999a)
Storage	Activity of endogenous enzymes in wheat samples which can subsequently influence AME value	Choct et al. (1995)
Animal-related factors	Variations in characteristics of different segments of the digestive tract which can affect nutrient digestibility and utilization by the animals	Maisonnier et al. (2001) De Verdal et al. (2010)
	Wet feeding	Scott (2002)

[1]Apparent metabolizable energy

Table 1.3 Prediction of AMEn of ingredients or complete diets through *in vitro* digestibility techniques in poultry

In vitro incubation conditions	Accuracy of prediction	Sample type	Disadvantages	Reference
Pepsin - 4hr Porcine intestinal fluid - 4hr	r = 0.93 RSD[1] = 145 kcal/kg	11 complete diets	Validated in adult roosters Different diets have different compositions and this *in vitro* technique may not be applicable to all diets	Clunies et al. (1984)
Pepsin - 4hr Pancreatin, bile salts, and enterokinase - 6hr	R^2 = 0.71 SEE[2] = 152 kcal/kg	71 complete diets	Validated in adult roosters Diets vary in their buffering capacity and one prediction equation may not accurately predict AME of all types of poultry diets	Valdes & Leeson (1992)
Pepsin – 2hr	R^2 = 0.75 RSD = 379 kcal/kg	52 samples of protein sources (oil seeds and oil seed by-products)	Validated in adult roosters Prediction equation was developed based on a combination of different ingredients varying in nutrient composition	Losada et al. (2010)

[1]Residual Standard Deviation

[2]Standard Error of Estimate

Chapter 2

Effects of corn source and exogenous enzyme products in broiler chicken diets. Growth performance and apparent ileal digestibility of energy[1]

2.1 Introduction

Corn is the main source of energy in poultry diets on a global scale (Ertl and Dale, 1997; Summers, 2001) and its inclusion rate in commercial diets can be up to 70% (Summers, 2001). According to the United States Department of Agriculture (2011), estimated global production of corn was 828.29 million tonnes for 2010/2011.

In spite of the general assumption that corn is relatively consistent in nutrient composition as compared to other cereal grains, there can be substantial variability in chemical content and available nutrient value of different sources of corn (Summers, 2001; Yin et al., 2002; Cowieson, 2005; Opapeju et al., 2007). As previously reviewed (Summers, 2001; Cowieson, 2005), various factors can influence nutritive value of corn. Cultivar (Opapeju et al., 2007) and environmental-related factors including location and growing and harvesting conditions (Leeson and Summers, 1976; Leeson et al., 1993; Scott et al., 2006; Opapeju et al., 2007) are amongst the factors that can greatly contribute to variability in the feeding value of this feedstuff. Determining this variability,

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especially with respect to energetic value, is of great help in formulating balanced diets in order to achieve optimal production performace at commercial operations (Yin et al., 2002; D'Alfonso, 2005; Opapeju et al., 2007).

Much of the available scientific information on exogenous enzyme application in poultry diets is related to high viscosity grains including wheat and barley which generally contain high levels of soluble non-starch polysaccharides (**NSP**). However, an increasing level of evidence indicates that the feeding value of corn-soy diets can also be increased by exogenous enzymes (Cowieson, 2005; Choct, 2006).

Corn and soybean meal, as two major ingredients in commercial poultry diets in many parts of the world, contain varying levels of different anti-nutritive factors (e.g., NSP and protease inhibitors) that can impede normal digestion and absorption processes of nutrients including carbohydrates and proteins in the digestive tract (Bach Knudsen 1997; Sheppy, 2001; Thorpe and Beal, 2001; Yu and Chung, 2004). Corn NSP content is lower than that of soybean meal (Cowieson and Adeola, 2005; Meng and Slominski, 2005), however, its contribution to the overall NSP level of the diet can be substantial due to its high inclusion rate in corn-soy diets. In the study of Meng and Slominski (2005), the total and water soluble NSP contents of corn were 76.3 and 6.4 mg/g, respectively. However, these values for soybean meal were 136.7 and 13.4 mg/g, respectively. This indicates that NSP-induced intestinal viscosity is generally not a problem in birds fed corn-soy based diets, but NSP compounds have the ability to prevent access to nutrients by encapsulating them (Gracia et al., 2003;

Cowieson, 2005; Choct, 2006; Slominski, 2011). The NSP-degrading enzymes (either with or without other enzyme activities) can enhance the access of endogenous enzymes to nutrients (e.g., starch granules) by releasing the nutrients from complex cell wall molecules (Yu and Chung, 2004; Leslie et al., 2007).

It has been estimated that there is about 400 to 450 kcal of energy per kg of diet not being digested when birds are fed with a typical corn-soy ration (Cowieson, 2010). A combination of undigested fat, protein, and starch contributes to this energy loss and use of exogenous enzymes can be a good strategy to make this energy available to birds (Cowieson, 2010). However, existing knowledge on the roles that exogenous enzyme products may play in enhancing the feeding value of corn-soy based diets in poultry is not only limited, but also inconsistent and as a result, more information is still required in this area of research (Ritz et al., 1995; Zanella et al., 1999; Gracia et al., 2009). The objectives of the current study were to investigate the effects of supplementation of different enzyme products on growth performance variables and ileal digestible energy (**IDE**) in the starter, grower, and finisher phases in broiler chickens fed corn-soy diets containing 3 corn sources of different geographical origins.

2.2 Materials and methods

2.2.1 Experimental design and diets

This experiment was approved by the Animal Care and Use Committee: Livestock of the University of Alberta and also met the guidelines of the Canadian Council on Animal Care (CCAC, 1993). A total of 3,600 one-d-old male broiler chicks (Ross 308 strain) were randomly assigned into groups of 30 chicks to 120 Specht pullet cages (53 × 59 × 44 cm, Specht Canada Inc., Stony Plain, AB,

Canada). The room temperature was initially set at 34 ºC, and was accordingly decreased to reach 18 ºC by day 39 which was the last day of the experiment. The lighting program was 23 hr light: 1hr dark per day and the birds had unrestricted access to feed and water throughout the feeding trial.

Three corn samples were obtained from the U.S.A and Canada. These samples were analyzed for DM, starch, CP, and oil contents before being used in the experimental diets. Results of chemical analyses of the 3 corn samples are shown in Table 2.1. In spite of different geographical origins, these corn samples had a similar nutrient content. A total of 15 experimental diets as 5 treatments for each of the three corn sources were prepared. A single source of soybean meal was used for the preparation of all 15 experimental dietary treatments.

The five dietary treatments used for each corn source were: Positive control (**PC**) with no exogenous enzymes and adequate in all nutrients according to the requirements set by the National Research Council (1994) and the primary breeder management guidelines for broiler chickens (Aviagen Inc. 2005); Negative control (**NC**) with no exogenous enzymes and a 3% reduction in calculated ME value relative to the PC diet; NC supplemented with 500 units/kg xylanase (Treatment X; Porzyme 9300, Danisco Animal Nutrition, Marlborough, Wiltshire, UK); NC supplemented with 300, 400, and 4,000 units/kg of xylanase, amylase, and protease, respectively (Treatment XAP; Avizyme 1505, Danisco Animal Nutrition, Marlborough, Wiltshire, UK); NC supplemented with 1,100 visco and 100 AGL units of xylanase and β-glucanase, respectively (Treatment XG; Rovabio Excel AP, Adisseo France S.A.S., Antony, France). The dietary

energy content was reduced in NC diets in order to investigate any effects that exogenous enzyme product addition may have on increasing nutrient and energy availability of these diets to the level of the respective PC diets (Zanella et al., 1999; Yu and Chung, 2004; Cowieson et al., 2006a; Cowieson and Ravindran, 2008b).

The ingredient composition and analyzed chemical content of the diets for the starter, grower, and finisher phases are given in Tables 2.2 and 2.3, respectively. Detailed information on the recovery of enzyme activities in all 15 dietary treatments is presented in Table 2.4 (recovery analyses were conducted by Danisco Animal Nutrition, Marlborough, Wiltshire, UK). The activity of β-glucanase enzyme was not measured in the experimental diets in the present study. As briefly described by Cowieson et al. (2006b), xylanase activity in the experimental diets was determined by following a modified method based on the Megazyme xylanase assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Amylase activity in the diets was assessed by using phadebas tablets (Megazyme International Ireland Ltd.), according to the method of Barnes and Blakeney (1974) and McCleary and Sheehan (1989). The activity of protease in the diet samples was tested by a modified Megazyme method (Megazyme International Ireland Ltd.), using pH 10 Tris/HCl as the extraction and assay buffers. The activities of the enzymes in the experimental rations of the present study were within expected limits. In the present study, enzyme activities (xylanase) were also recovered from one of the control diets (PC diet of Corn 1 with no exogenous xylanase being added). The reasons for the presence of

xylanase in the control diet are not clear. The diets were fed to the birds in a mash form. Celite (Celite Corporation, World Minerals Inc., Lompoc, CA) was included in all diets at 1% as an indigestible marker to determine dry matter (**DM**) digestibility and IDE values.

2.2.2 Growth performance and digestibility measurements

Each diet was fed to 8 cages of 30 chicks (initially 240 chicks per diet) in 3 phases as the starter (0 to11 d of age), grower (12 to 28 d of age), and finisher (29 to 39 d of age). Body weight and feed intake were recorded and feed conversion ratio (FCR) was calculated for each cage at the end of each of the starter, grower, and finisher phases. Cage mortality (number and weight of dead birds within each cage) was recorded daily throughout the experiment and FCR was corrected accordingly at the time of calculating growth performance variables.

At days 11, 28, and 39 of age, birds (15 birds from each cage at d 11, 9 birds at d 28, and all remaining birds at d 39) were euthanized by cervical dislocation and contents of the ileum (from Meckel's diverticulum to the ileo-cecal junction) were collected in plastic bags, pooled within each cage, and frozen immediately. Pooled digesta samples were subsequently freeze-dried and ground for laboratory analyses (Garcia et al., 2007). Diets and ileal digesta samples were analyzed for DM, acid-insoluble ash (**AIA**), and gross energy (**GE**).

2.2.3 Chemical analyses

The DM (method 934.01; AOAC, 2005) and AIA (McCarthy et al., 1974) contents of experimental diets and ileal digesta samples were determined. The GE of samples was measured by bomb calorimetry using an adiabatic calorimeter

(AC-300, Leco Corp., St. Joseph, MI) calibrated with benzoic acid (Gunawardena et al., 2010).

The apparent ileal digestibility of dietary DM and also IDE of the diets were calculated according to the following formulas (Olukosi et al., 2007b):

Nutrient digestibility (%) =

$$\{1-[(AIA_{feed}/AIA_{digesta}) \times (Nutrient_{digesta}/Nutrient_{feed})]\} \times 100$$

$$IDE\ (kcal/kg) = GE_{feed} - [GE_{digesta} \times (AIA_{feed}/AIA_{digesta})]$$

The IDE is a measure of the amount of energy being absorbed by the bird up to the ileum, minimizing the confounding effects of hindgut microbiota on energy measurement using excreta samples (Olukosi et al., 2007b).

2.2.4 Statistical analyses

The experiment was conducted as a completely randomized design and growth performance and digestibility data were analyzed by ANOVA (Kuehl, 2000) using the GLM procedure of SAS (SAS Institute, 2002) to examine the effect of dietary treatments (SAS Institute, 2002, Olukosi et al., 2007b). These analyses were performed for each corn sample within each production phase (i.e., starter, grower, and finisher) with 8 observations (replicates) for each dietary treatment. The cage was the experimental unit and least-squares means were compared using orthogonal contrasts (SAS Institute, 2002, Olukosi et al., 2007b). Differences were considered significant at $P \leq 0.05$.

2.3 Results

2.3.1 Growth performance variables

Corn 1: Dietary treatments had no effects ($P > 0.05$) on performance variables in the starter and finisher phases (Table 2.5). In the grower phase, birds fed the XAP diet had higher feed intake ($P = 0.050$) than the NC diet. Birds receiving the X diet had higher FCR ($P = 0.039$) compared to the NC fed group.

Corn 2: Diets had no effect ($P > 0.05$) on performance of birds in the starter phase except for a higher feed intake ($P = 0.040$) in birds fed the XAP diet relative to the NC birds (Table 2.6). There was also no effect ($P > 0.05$) of diets in the grower phase. In the finisher phase, birds fed with the XAP diet had higher weight gain ($P = 0.036$) compared to the NC diet. However, enzyme treatments had no effect on feed intake and FCR relative to the NC diet.

Corn 3: In the starter phase, birds fed the XG diet had higher feed intake ($P = 0.024$) compared to the NC diet (Table 2.7). There was no effect of diets on BW gain and FCR. Dietary treatments had no effects ($P > 0.05$) on performance variables in the grower and finisher phases.

2.3.2 Digestibility measures

Corn 1: In the starter phase, digestibility of DM and IDE were not different ($P > 0.05$) in the NC and PC diets (Table 2.8) and enzyme inclusion had no effect ($P > 0.05$) on digestibility measures. In the grower phase, digestibility of the NC diet was lower ($P < 0.05$) than the PC diet, however, supplementation of enzyme products did not result in any increases in ileal digestibility compared to the NC diet. In the finisher phase, the NC diet again had higher digestibility values than the PC diet and addition of product X reduced ($P = 0.014$) DM digestibility compared to the NC diet.

Corn 2: In the starter phase, diets had no effects ($P > 0.05$) on digestibility of DM and IDE (Table 2.9). In the grower phase, supplementation of diets with each of the enzyme products increased ($P < 0.05$) digestibility values compared to the NC diet. The increases in digestibility of DM were 13.0, 11.2, and 10.8% for the X, XAP or XG diets, respectively. The IDE was also increased by 8.0, 7.1, and 6.3% as a result of inclusion of products X, XAP or XG, respectively. In the finisher phase, only XAP supplementation increased DM digestibility (by 4%) and IDE (by 3.2%) compared to the NC diet.

Corn 3: There was no effect of diets ($P > 0.05$) on DM digestibility and IDE in the starter and grower phases (Table 2.10). In the finisher phase, supplementation of XG increased DM digestibility (5.9%) and IDE (4%) compared to the NC diet.

2.4 Discussion

The effects of dietary treatments on BW gain, feed intake, and FCR were generally not significant. In cases of significant differences, they lacked consistency and in some cases, enzyme addition had a negative impact. The lack of responses in performance variables to exogenous enzyme treatments has also been reported in other studies in which broiler chickens were fed with corn-soy diets. In the study of Kocher et al. (2003), none of the exogenous enzyme products tested (including xylanase and xylanase, amylase, plus protease) had any effects on the performance of broiler chickens. Olukosi et al. (2007b) also observed that addition of a cocktail of xylanase, amylase, and protease had no effects on BW gain and feed efficiency in broiler chickens fed corn-soy diets for 21 d of age. West et al. (2007) conducted 3 separate experiments and observed

that addition of an enzyme product containing xylanase and β-glucanase had no significant impact on BW and feed conversion in broilers fed corn-soy diets for up to 49 days of age.

One explanation for the lack of effects of enzyme treatments on growth performance variables in the present study could be that the extent of reduction in calculated metabolizable energy content of NC diets (a calculated 3% reduction relative to the PC diets) was not substantial enough to produce detectable effects on growth performance (Troche et al., 2007; West et al., 2007). A greater reduction could have possibly elicited greater enzyme responses with respect to growth performance variables (Troche et al., 2007; West et al., 2007).

The lack of effects on growth performance does not necessarily mean that enzyme products are not able to work on their specific substrates (Cowieson and Adeola, 2005; Olukosi et al., 2007a) as products X, XAP, and XG had some transient effects on DM digestibility and IDE in the present study. Meng and Slominski (2005) suggested that the effects of cell-wall degrading enzymes on growth performance may be small and not always detectable under experimental settings. However, these small effects can be associated with economical benefits at large commercial poultry farms (Meng and Slominski, 2005).

Bird responses to enzyme product inclusions were mainly observed in the grower phase (at day 28) of the present study. It is generally expected to observe greater responses to exogenous enzyme products in younger animals as endogenous enzymatic activities in the digestive tract are limited and this can limit efficiency of the digestive tract at the early stages of life (Olukosi et al.,

2007b). Supplementing exogenous enzymes at early ages can enhance digestibility of nutrients through increasing enzyme presence or activity in the digestive tract. On the other hand, exogenous enzymes can also decrease the requirements for the synthesis of endogenous enzymes so that more nutrients can be devoted to the growth of birds (Olukosi et al., 2007b). However, results of the present study did not exactly follow this pattern as there were no effects of enzyme products on ileal digestibility parameters in the starter phase. There were no differences in ileal digestibility of DM and IDE between PC and NC diets in the starter phase and in some cases, digestibility values of NC diet were even higher than the PC diet. The reasons for these observations are not clear, however, this situation might have played a major role as to why we did not observe any effects of enzyme treatments in this phase.

Our findings in the grower phase are in accordance with Gracia et al. (2003) who also reported positive effects of dietary supplementation of α-amylase on nutrient digestibility at day 28 in male broiler chickens fed with corn-soy diets. This could be an indication that the digestive tract of broiler chickens might not be completely developed by 28 days of age and nutrient digestibility can still be further enhanced by exogenous enzyme products (Gracia et al., 2003).

The effects of enzyme products observed in the present study were dependent on corn source, enzyme product profile, and age of the birds. The greatest positive responses to enzyme product addition were in the grower phase and were mostly observed in birds fed Corn 2 diets as digestibility parameters were significantly higher than the NC diet. As stated earlier, 3 corn samples used in the present study

had a similar nutrient content, but supplementation of Corn 2 diets with enzyme products was associated with greater responses on digestibility.

As reviewed by Cowieson (2010), the nutritional quality of the diet is probably the most important factor which influences responses to an enzyme product. Responses to exogenous enzyme product addition are expected to be greater in diets with lower nutritional quality (Wyatt et al., 1999; Kocher et al., 2003; Cowieson and Ravindran, 2008b; Zhou et al., 2009; Cowieson et al., 2010). Zhou et al. (2009) reported that addition of xylanase, amylase, and protease was associated with greater effects on increasing AME of corn-soy diets with lower energy contents compared to diets with higher energy levels fed to broiler chickens. These observations may explain as to why greater responses to enzyme supplementation were seen in birds fed Corn 2 diets.

There are several suggested mechanisms of action of exogenous enzyme products in corn-soy diets (Cowieson, 2005). Digestibility of starch in the small intestine can be increased by the addition of exogenous enzyme products into corn-soy diets, leading to enhanced energy availability of diets to birds (Zanella et al., 1999; Yu and Chung, 2004; Meng and Slominski, 2005). Leslie et al. (2007) suggested that exogenous glucanase can increase IDE value through enhancing starch digestion via increases in the amylase access to the starch granules. In another study, D’Alfonso (2005) concluded that that exogenous xylanase and protease can work together in order to provide a proper condition for the action of exogenous amylase on the starch, which can result in increased ileal starch digestibility and IDE values. Our observations also suggest that enzyme products

used in the present study (mixture of enzyme activities including amylase, protease, xylanase, and β-glucanase) had transient effects on IDE of corn-soy diets, possibly through enhancing digestibility of dietary starch in the small intestine.

It is also suggested that exogenous enzymes can increase the access to entrapped nutrient components through destroying some fractions of the cell walls of ingredients (Kocher et al., 2003; D'Alfonso, 2005; Leslie et al., 2007). As stated earlier, NSP-induced viscosity is generally not a problem in corn-soy based diets, but NSP compounds have the ability to negatively influence the access of endogenous enzymes to nutrients by encapsulating the nutrients in the cell wall structures in corn (Gracia et al., 2003; Cowieson, 2005; Choct, 2006; Slominski, 2011). Although exogenous xylanase is able to release the nutrients exisiting in the cell wall structures, this action might not result in an effective response if xylanase is not accopmained by other enzyme activities such as protease and amylase (Cowieson, 2005). Cowieson et al. (2010) also proposed that synergism between xylanase and glucanase can degrade the cell wall structures more efficiently, resulting in the release of nutrients from the cells. This pattern can also apply to our findings in the present study as supplementation of enzymes with a mixture of activities were associated with transient effects on ileal digestibility parameters and this could have been resulted from increased exposure of dietary nutrients to endogenous enzymes present in the diegstive tract.

As reviewed by Cowieson et al. (2006b), one of the main challenges with respect to enzyme product supplementation is that enzyme addition may not

always lead to enhancement of growth performance or digestibility of nutrients as it was observed in the present study as well. In general, the inconsistencies in the results of exogenous enzyme studies conducted with corn-soy diets can be attributed to a wide array of factors (Cowieson et al., 2006b). Some of these factors may include differences in the types and activities of enzymes (Gracia et al., 2003), the types of bacteria or fungi being used to produce enzyme products (Gracia et al., 2003; Olukosi et al., 2007a), inclusion level of the enzymes into the diets (Cowieson and Ravindran, 2008a), addition of a single or a mixture of enzyme activities (Cowieson and Adeola, 2005; Cowieson et al. 2006b; Olukosi et al., 2007b), nutritional quality of dietary ingredients (Douglas et al., 2000; Cowieson et al. 2006b; Cowieson and Ravindran, 2008b; Zhou et al., 2009), and form of the diet as mash, crumble, or pellet (Gracia et al., 2003; Yang et al., 2010).

Our observations in terms of effects of product XG on ileal digestibility parameters were interesting as there is limited information available on the effects of xylanase and β-glucanase in corn-soy diets fed to broiler chickens. The vast majority of studies of xylanase and β-glucanase have been conducted in diets containing high viscosity grains such as wheat and barley (Cowieson, 2005; West et al., 2007). Although β-glucanase activity was not measured in the experimental diets in the present study, our observations showed that product XG (xylanase and β-glucanase) had transient effects on IDE in corn-soy diets. Leslie et al. (2007) reported that effects of glucanase on increasing IDE value of corn might be a result of degradation of the cell wall which subsequently increases the access of

endogenous enzymes (i.e., amylase) to starch granules within the endosperm. However, it is not clearly known how glucanase can act on soybean meal (Leslie et al., 2007). As indicated by Cowieson et al. (2010), more studies are still required to further investigate the mechanisms of action of a combination of xylanase and β-glucanase in corn-soy diets in broiler chickens.

In conclusion, effects of enzyme treatments on IDE were not consistent and varied depending on corn source, enzyme product profile, and dietary phase. Enzyme products elicited the greatest responses in birds fed Corn 2 diets in the grower phase mainly. Factors related to diet (e.g., availability of substrates) and birds (e.g., enzymatic activities in the digestive tract) may have limited nutrient digestibility (i.e., Corn 2 diets in the grower phase of this study), and this limitation has provided favorable conditions for exogenous enzyme products to specifically work on their substrates (Cowieson et al., 2006a). Using exogenous enzymes that can target a broad range of substrates may enhance responses in corn-soy diets and this can be a pragmatic approach in poultry feeding (Cowieson et al., 2010). According to Choct (2006), a better understanding of enzyme-substrate specificity, the gut microbiota, and immune system activity would increase our knowledge of how exogenous enzyme products can enhance nutritive value of corn-soy diets in broiler chickens.

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Table 2.1 Chemical analyses of corn samples (%) - DM basis

Source	Harvest/origin	DM	Starch	CP	Ether extract
Corn 1	2005/USA	90.3	70.1	8.5	4.1
Corn 2	2005/USA	88.6	69.5	9.5	3.7
Corn 3	2005/Canada	91.1	69.4	8.2	3.8

Table 2.2 Composition of positive and negative control diets (kg/tonne)

Ingredients(kg/tonne)	Starter		Grower		Finisher	
	PC[1]	NC[2]	PC	NC	PC	NC
Corn	607.6	630.5	630.4	653.2	639.5	662.2
Vegetable oil	25.9	6.7	37.9	18.7	51.7	32.5
Soybean Meal (48%)	300.9	297.2	267.6	263.8	247.1	243.4
Calcium carbonate	13.9	14.0	13.7	13.8	14.6	14.7
Dicalcium phosphate	15.2	15.2	14.4	14.4	11.9	11.9
Sodium chloride	4.2	4.2	3.9	3.9	3.7	3.7
L – Lysine	4.4	4.5	4.3	4.4	3.8	3.9
DL- Methionine	4.2	4.1	4.0	4.0	3.8	3.8
L-Threonine	0.0	0.0	0.0	0.0	0.2	0.2
Vitamin-mineral premix[3]	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride[4]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin E 5,000 IU/kg	3.0	3.0	3.0	3.0	3.0	3.0
Product X[5]	0.0	0.1	0.0	0.1	0.0	0.1
Product XAP[6]	0.0	0.2	0.0	0.2	0.0	0.2
Product XG[7]	0.0	0.05	0.0	0.05	0.0	0.05
Celite	10.0	10.0	10.0	10.0	10.0	10.0
Coccidiostat[8]	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient content						
Protein (%)	20.5	20.5	19.0	19.0	18.0	18.0
Lys (%)	1.4	1.4	1.3	1.3	1.2	1.2
Met (%)	0.73	0.73	0.70	0.70	0.66	0.66
Met + Cys (%)	1.05	1.05	1.00	1.00	0.95	0.95
ME (kcal/kg)	3,050	2,950	3,150	3,050	3,250	3,150
Ca (%)	0.9	0.9	0.87	0.87	0.85	0.85
Available P (%)	0.4	0.4	0.38	0.38	0.33	0.33

[1]PC= Positive control

[2]NC=Negative control

[3]Vitamin/mineral premix provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 ICU; vitamin E, 35 IU; menadione, 2.0 mg; D-pantothenic acid, 14 mg; riboflavin, 5.0 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18; iodine, 0.5 mg; manganese, 70 mg; copper, 8.5 mg; zinc, 80 mg; selenium, 0.1 mg; iron, 100 mg

[4]Provided 100 mg of choline per kilogram of diet

[5]Product X= 0.1g /kg diet (Porzyme 9300, provided 500 units of xylanase per kilogram of diet)

[6]Product XAP= 0.2g/kg diet (Avizyme 1505, provided 300, 400, 4000 units of xylanase, amylase, and protease respectively per kilogram of diet)

[7]Product XG= 0.05g/kg diet (Rovabio Excel AP, provided 1,100 visco and 100 AGL units of xylanase and β glucanase, respectively per kilogram of diet)

[8]Amprol, Huvepharma Inc., Peachtree City, GA

Table 2.3 Analyzed chemical composition of the experimental diets (%) - DM basis

Item	Corn 1 diets			Corn 2 diets			Corn 3 diets		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
DM	88.9	89.3	89.8	89.2	89.5	89.8	89.6	89.8	90.6
CP	23.4	20.9	20.5	23.8	22.3	20.9	23.6	21.7	19.7
Lys	1.20	1.14	1.02	1.23	1.17	1.09	1.23	1.15	0.98
Met	0.59	0.51	0.49	0.59	0.53	0.51	0.57	0.54	0.50
Cys + Met	0.85	0.74	0.74	0.87	0.79	0.78	0.82	0.80	0.74
Ca	1.08	0.90	1.10	1.07	1.16	1.29	1.35	1.29	1.20
P (total)	0.80	0.78	0.72	0.81	0.78	0.69	0.86	0.80	0.73

Table 2.4 Recovery of xylanase, amylase, and protease activities in 15 experimental diets (Units/kg)[1]

Diets	Xylanase	Amylase	Protease
Corn 1			
PC[2]	578	<100	<100
NC[3]	<100	<100	NA[4]
NC+ X[5]	575	<100	<100
NC+ XAP[6]	303	1,767	1,648
NC+ XG[7, 8]	3,530	<100	<100
Corn 2			
PC	105	<100	<100
NC	<100	<100	<100
NC+ X	517	<100	<100
NC+ XAP	425	1,923	1,241
NC+ XG	2,739	<100	<100
Corn 3			
PC	<100	<100	<100
NC	<100	<100	<100
NC+ X	564	<100	<100
NC+ XAP	345	2,243	1,609
NC+ XG	3,360	<100	<100

[1] Danisco Animal Nutrition, Marlborough, Wiltshire, UK
[2] Positive control
[3] Negative control
[4] Not available
[5] Product X= Xylanase (500 units of xylanase per kg of diet)
[6] Product XAP = Xylanase, amylase, and protease (300, 400, 4000 units of xylanase, amylase, and protease respectively per kg of diet)
[7] Product XG = Xylanase + β-glucanase (1,100 visco and 100 AGL units of xylanase and β-glucanase, respectively per kg of diet)
[8] β-glucanase activity was not measured in the experimental diets

Table 2.5 Effects of dietary treatments on performance variables of birds fed Corn 1 diets[1]

Diets	BW gain (g/d/ bird)	Feed intake (g/d/bird)	FCR[2] (g feed: g gain)
Starter (0-11 d)			
PC[3]	12.7	19.1	1.52
NC[4]	12.7	20.5	1.61
NC+X[5]	13.0	20.2	1.56
NC+XAP[6]	13.0	19.7	1.52
NC+XG[7]	13.0	19.6	1.52
SEM	0.56	0.64	0.05
P-value[8]			
PC vs. NC	0.962	0.132	0.233
NC vs. NC+X	0.683	0.730	0.482
NC vs. NC+XAP	0.706	0.387	0.202
NC vs. NC+XG	0.694	0.344	0.233
Grower (12-28 d)			
PC	37.9	63.5	1.68
NC	38.4	61.0	1.59
NC+X	39.6	67.1	1.69
NC+XAP	40.3	67.4	1.67
NC+XG	38.6	63.6	1.65
SEM	1.10	2.25	0.03
P-value			
PC vs. NC	0.749	0.426	0.079
NC vs. NC+X	0.474	0.063	0.039
NC vs. NC+XAP	0.242	0.050	0.102
NC vs. NC+XG	0.936	0.410	0.199
Finisher (29-39 d)			
PC	66.6	131.6	1.99
NC	63.5	126.5	2.00
NC+X	63.4	134.8	2.12
NC+XAP	64.1	130.3	2.07
NC+XG	63.9	125.9	1.97
SEM	2.55	5.71	0.09
P-value			
PC vs. NC	0.397	0.531	0.956
NC vs. NC+X	0.988	0.311	0.367
NC vs. NC+XAP	0.861	0.648	0.592
NC vs. NC+XG	0.906	0.940	0.810

[1]All means are average of 8 observations per treatment
[2]Feed conversion ratio
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Table 2.6 Effects of dietary treatments on performance variables of birds fed Corn 2 diets[1]

Diets	BW gain (g/d/bird)	Feed intake (g/d/bird)	FCR[2] (g feed: g gain)
Starter (0-11 d)			
PC[3]	13.8	20.2	1.47
NC[4]	13.1	19.1	1.46
NC+X[5]	13.4	20.0	1.50
NC+XAP[6]	13.7	20.5	1.50
NC+XG[7]	13.2	19.8	1.50
SEM	0.41	0.46	0.03
P-value [8]			
PC vs. NC	0.228	0.088	0.885
NC vs. NC+X	0.654	0.179	0.374
NC vs. NC+XAP	0.329	0.040	0.405
NC vs. NC+XG	0.814	0.269	0.330
Grower (12-28 d)			
PC	41.5	66.3	1.6
NC	39.1	64.3	1.6
NC+X	40.3	64.8	1.6
NC+XAP	39.7	63.7	1.6
NC+XG	39.3	65.3	1.7
SEM	1.10	1.99	0.02
P-value			
PC vs. NC	0.143	0.485	0.182
NC vs. NC+X	0.464	0.853	0.290
NC vs. NC+XAP	0.702	0.815	0.206
NC vs. NC+XG	0.898	0.731	0.645
Finisher (29-39 d)			
PC	68.4	129.8	1.90
NC	63.3	136.2	2.15
NC+X	64.1	130.3	2.04
NC+XAP	67.7	133.6	1.97
NC+XG	67.4	143.0	2.14
SEM	1.44	4.30	0.06
P-value			
PC vs. NC	0.016	0.299	0.009
NC vs. NC+X	0.708	0.338	0.208
NC vs. NC+XAP	0.036	0.664	0.058
NC vs. NC+XG	0.053	0.276	0.871

[1]All means are average of 8 observations per treatment
[2]Feed conversion ratio
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Table 2.7 Effects of dietary treatments on performance variables of birds fed Corn 3 diets [1]

Diets	BW gain (g/d/bird)	Feed intake (g/d/bird)	FCR[2] (g feed: g gain)
Starter (0-11 d)			
PC[3]	13.5	20.1	1.51
NC[4]	12.8	19.1	1.50
NC+X[5]	13.2	19.8	1.51
NC+XAP[6]	12.8	19.8	1.56
NC+XG[7]	13.9	20.9	1.50
SEM	0.57	0.52	0.05
P-value [8]			
PC vs. NC	0.343	0.211	0.849
NC vs. NC+X	0.591	0.356	0.939
NC vs. NC+XAP	0.963	0.331	0.364
NC vs. NC+XG	0.167	0.024	0.985
Grower (12-28 d)			
PC	40.3	67.2	1.67
NC	38.5	62.5	1.62
NC+X	39.0	64.9	1.66
NC+XAP	39.6	64.9	1.64
NC+XG	38.4	64.0	1.66
SEM	0.98	2.00	0.03
P-value			
PC vs. NC	0.211	0.106	0.300
NC vs. NC+X	0.747	0.418	0.356
NC vs. NC+XAP	0.431	0.415	0.772
NC vs. NC+XG	0.943	0.615	0.371
Finisher (29-39 d)			
PC	67.2	132.4	1.98
NC	65.1	129.8	2.01
NC+X	69.3	136.8	1.98
NC+XAP	66.6	131.1	1.97
NC+XG	66.1	129.6	1.97
SEM	2.33	3.77	0.07
P-value			
PC vs. NC	0.518	0.624	0.787
NC vs. NC+X	0.206	0.187	0.746
NC vs. NC+XAP	0.676	0.821	0.674
NC vs. NC+XG	0.766	0.968	0.695

[1]All means are average of 8 observations per treatment
[2]Feed conversion ratio
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Table 2.8 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 1 diets[1] (DM basis)

Diets	DM (%)	IDE[2] (kcal/kg)
Starter (0-11 d)		
PC[3]	45.3	2,340
NC[4]	50.4	2,578
NC+X[5]	48.8	2,490
NC+XAP[6]	52.3	2,622
NC+XG[7]	51.2	2,593
SEM	2.00	81.15
P-value [8]		
PC vs. NC	0.090	0.055
NC vs. NC+X	0.570	0.451
NC vs. NC+XAP	0.516	0.709
NC vs. NC+XG	0.792	0.897
Grower (12-28 d)		
PC	69.8	3,341
NC	66.0	3,223
NC+X	67.1	3,292
NC+XAP	67.7	3,294
NC+XG	66.5	3,240
SEM	0.79	36.51
P-value		
PC vs. NC	0.002	0.029
NC vs. NC+X	0.343	0.188
NC vs. NC+XAP	0.146	0.176
NC vs. NC+XG	0.641	0.743
Finisher (29-39 d)		
PC	64.7	3,238
NC	72.5	3,513
NC+X	67.4	3,362
NC+XAP	69.8	3,437
NC+XG	70.9	3,504
SEM	1.41	54.87
P-value		
PC vs. NC	0.001	0.002
NC vs. NC+X	0.014	0.061
NC vs. NC+XAP	0.174	0.335
NC vs. NC+XG	0.399	0.907

[1]All means are average of 8 observations per treatment
[2]Ileal digestible energy
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Table 2.9 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 2 diets[1] (DM basis)

Diets	DM (%)	IDE[2] (kcal/kg)
Starter (0-11 d)		
PC[3]	57.2	2,805
NC[4]	57.0	2,821
NC+X[5]	58.2	2,865
NC+XAP[6]	57.0	2,802
NC+XG[7]	55.4	2,739
SEM	1.58	63.30
P-value [8]		
PC vs. NC	0.948	0.865
NC vs. NC+X	0.614	0.620
NC vs. NC+XAP	0.986	0.830
NC vs. NC+XG	0.462	0.360
Grower (12-28 d)		
PC	63.1	3,113
NC	57.6	2,956
NC+X	65.1	3,193
NC+XAP	64.1	3,167
NC+XG	63.8	3,158
SEM	0.85	33.90
P-value		
PC vs. NC	<0.001	0.002
NC vs. NC+X	<0.001	<0.001
NC vs. NC+XAP	<0.001	<0.001
NC vs. NC+XG	<0.001	0.002
Finisher (29-39 d)		
PC	70.1	3,375
NC	69.3	3,385
NC+X	71.2	3,465
NC+XAP	72.1	3,493
NC+XG	67.4	3,334
SEM	0.89	36.09
P-value		
PC vs. NC	0.542	0.837
NC vs. NC+X	0.140	0.128
NC vs. NC+XAP	0.036	0.043
NC vs. NC+XG	0.142	0.326

[1]All means are average of 8 observations per treatment
[2]Ileal digestible energy
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Table 2.10 Effects of dietary treatments on ileal DM and energy digestibility in birds fed Corn 3 diets[1] (DM basis)

Diets	DM (%)	IDE[2] (kcal/kg)
Starter (0-11 d)		
PC[3]	52.5	2,601
NC[4]	51.9	2,606
NC+X[5]	49.9	2,498
NC+XAP[6]	53.3	2,665
NC+XG[7]	53.4	2,675
SEM	1.86	75.60
P-value [8]		
PC vs. NC	0.817	0.961
NC vs. NC+X	0.467	0.320
NC vs. NC+XAP	0.582	0.585
NC vs. NC+XG	0.553	0.524
Grower (12-28 d)		
PC	63.1	3,010
NC	62.3	2,979
NC+X	63.9	3,030
NC+XAP	62.1	2,913
NC+XG	60.7	2,883
SEM	1.19	53.26
P-value		
PC vs. NC	0.650	0.681
NC vs. NC+X	0.355	0.508
NC vs. NC+XAP	0.882	0.379
NC vs. NC+XG	0.329	0.207
Finisher (29-39 d)		
PC	69.6	3,360
NC	67.3	3,327
NC+X	70.1	3,433
NC+XAP	69.1	3,362
NC+XG	71.3	3,462
SEM	1.17	45.50
P-value		
PC vs. NC	0.169	0.603
NC vs. NC+X	0.094	0.107
NC vs. NC+XAP	0.282	0.586
NC vs. NC+XG	0.020	0.043

[1]All means are average of 8 observations per treatment
[2]Ileal digestible energy
[3]Positive control
[4]Negative control
[5]Product X= Xylanase
[6]Product XAP = Xylanase, amylase, and protease
[7]Product XG = Xylanase + β-glucanase
[8]Significance level ($P \leq 0.05$)

Chapter 3

Effects of corn source and exogenous enzyme products in broiler chicken diets. Apparent ileal digestibility of crude protein and amino acids[1]

3.1 Introduction

Although corn is a highly digestible source of energy, it also contributes other nutrients including crude protein (**CP**) and amino acids (**AA**) to diets (Lilburn et al., 1991; Summers, 2001; Opapeju et al., 2007). Depending on the inclusion rate of corn, this contribution can be more than 20% of the total CP content of commercial poultry rations (Lilburn et al., 1991; Summers, 2001). This contribution, however, is not sufficient to meet bird requirements and other sources of protein of vegetable (e.g., soybean meal) or animal origins (e.g., fish meal), and also crystalline AA often must be added to corn-based diets for broiler chickens (Sauberlich et al., 1953; Lewis et al., 1982; Thorpe and Beal, 2001; Leeson and Summers, 2005). Among protein sources, soybean meal is the most commonly used ingredient for commercial poultry diets on a global basis (Waldroup, 2002; Coon, 2005).

Reducing feed costs at commercial poultry farms is a constant concern for the industry and current upward trend in the price of feedstuffs has generated an increasing interest in using exogenous enzyme products in corn-soy diets (Cowieson and Ravindran, 2008a; Cowieson, 2010).

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[1]A version of this chapter has been submitted to *Poultry Science* (Yegani, M and D.R. Korver. 2012. Effects of corn source and exogenous enzyme products in broiler chicken diets. Apparent ileal digestibility of CP and amino acids. *Revision Submitted*).

Several studies have shown that addition of exogenous enzyme products containing non- starch polysaccharides (**NSP**)-degrading enzymes, either with or without other enzyme activities, to corn-soy diets can increase digestibility of CP (Zanella et al. 1999; D'Alfonso, 2005; Cowieson and Ravindran, 2008b) and AA (Zanella et al. 1999; Cowieson et al., 2006a; Cowieson and Ravindran, 2008b). Supplementing corn-soy based broiler chicken diets with an enzyme product containing xylanase, amylase, and protease resulted in a 2.9% increase in the ileal digestibility of CP, although this increase did not occur to the same extent for all AA (Zanella et al., 1999). Cowieson and Ravindran (2008b) also reported that addition of a mixture of enzymes (xylanase, amylase, and protease) to corn-soy diets in broiler chickens caused an average increase of 2.8% in the ileal digestibility of AA, with 0.44 and 9.1% as the lowest and highest increases for Met and Cys, respectively. In another study, Rutherfurd et al. (2007) used an enzyme cocktail containing xylanase, amylase and β-glucanase in corn-soy diet in broilers. In the diet without enzymes, Cys (58.9%) and Met (88.8%) had the lowest and highest ileal digestibility, respectively. There was a substantial increase in the apparent ileal digestibility of all AA as a result of xylanase, amylase, and β-glucanase addition. The Met had the lowest increase (3.2%) in digestibility due to this enzyme addition compared to Cys (12%) and other AA.

It has been reported that carbohydrases (either with or without other enzyme activities) can also enhance ileal digestibility of AA of diets for poultry (Cowieson and Bedford, 2009). However, the factors affecting these responses are not clear, nor are the responses to different enzyme combinations. The objectives

of the current study were to investigate effects of supplementation of different commercial enzyme products on apparent ileal digestibility of CP and essential and non-essential AA of the starter, grower, and finisher diets formulated using 3 sources of corn of different geographical origins.

3.2 Materials and methods

3.2.1 Experimental design and diets

A total of 3,600 one-d-old male broiler chicks (Ross 308 strain) were randomly assigned into groups of 30 chicks to each of 120 Specht pullet cages (53 × 59 × 44 cm, Specht Canada Inc., Stony Plain, AB, Canada). Three corn samples were obtained from the U.S.A and Canada. These samples were analyzed for DM, starch, protein, and oil contents before being used for mixing the experimental diets. Although attempts were made to increase variability among the corn samples by sourcing them from different geographical regions, the analyzed chemical compositions were similar (Chapter 2, Table 2.1). A single source of soybean meal was used for the preparation of all 15 experimental dietary treatments. All corn samples were received as whole grain and ground under the same conditions. Ingredient composition and analyzed chemical content of the diets and also recovered exogenous enzyme activities in the diets are reported in Chapter 2.

The five dietary treatments used for each corn source were: Positive control (**PC**) with no exogenous enzymes and adequate in all dietary nutrients according to the requirements set by the National Research Council (1994) and the primary

breeder management guide for broiler chickens (Aviagen Inc. 2005); Negative control (**NC**) with no exogenous enzymes and a 3% reduction in calculated ME value relative to the PC diet; NC supplemented 500 units/kg xylanase (Treatment X; Porzyme 9300, Danisco Animal Nutrition, Marlborough, Wiltshire, UK); NC supplemented with 300, 400, and 4,000 units/kg of xylanase, amylase, and protease, respectively (Treatment XAP; Avizyme 1505, Danisco Animal Nutrition, Marlborough, Wiltshire, UK); NC supplemented with 1,100 visco and 100 AGL units of xylanase and β-glucanase, respectively (Treatment XG; Rovabio Excel AP, Adisseo France S.A.S., Antony, France). Experimental diets were fed as a starter from d 0 to d 11, a grower from d 12 to d 28, and a finisher from d 29 to d 39 of age (Chapter 2).

3.2.2 Digestibility measurements

At days 11, 28, and 39 of age (corresponding to the end of the starter, grower, and finisher phases, respectively), birds (15 birds from each cage at d 11, 9 birds at d 28, and the remainder of the birds at d 39 of age) were euthanized by cervical dislocation and contents of the ileum (from the Meckel's diverticulum to the ileo-cecal junction) were collected into plastic bags, pooled (within each cage), and frozen immediately. Pooling of digesta samples within each cage was necessary in order to obtain sufficient amount of sample from each cage for chemical analyses. Pooled digesta samples were subsequently freeze-dried and ground for laboratory analyses (Garcia et al., 2007). Diets and ileal digesta samples were analyzed for dry matter (**DM**), acid-insoluble ash (**AIA**), CP, and essential and non-essential AA.

3.2.3 Chemical analyses

The DM (method 934.01; AOAC, 2005) and AIA (McCarthy et al., 1974) contents of experimental diets and ileal digesta samples were determined. Nitrogen content of samples was determined by combustion with an automatic nitrogen analyzer (Leco TruSpec CN, Leco Corp., St. Joseph, MI), and then multiplied by 6.25 to calculate CP content of each sample (method 968.06; AOAC, 2005).

The AA contents of diets and digesta samples were determined by hydrolyzing approximately 100 mg of each sample with 6M HCl for 24 h at 110 °C to allow the release of AA from protein molecules. AA were subsequently separated and quantified on a Varian HPLC instrument (Varian Prostar 210 pump and 410 autosampler, Varian Inc., Palo Alto, CA) and a Varian Fluorichrom fluorescence detector. A reverse phase column (Supelcosil 3 micron LC-18, 4.6 × 150 mm) was used for separation of AA. The samples were derivatized with o-Phthaldialdehyde before injection. Beta-amino-n-butyric acid and ethanolamine were used as internal standards. The acquisition and integration of chromatograms was done by Galaxie software (Galaxie Chromatography Data System, Varian Inc., Palo Alto, CA). In the standard hydrolysis procedure, all AA were quantified except Cys, Met, Trp, and Pro (Sedgwick et al., 1991; method 994.12; AOAC, 2005; Cowieson and Ravindran, 2008b).

The Cys and Met were determined as cysteic acid and methionine sulfone through the performic acid oxidation method. In this method, samples went through performic acid oxidation prior to HCL hydrolysis. Cold performic acid (a

1:9 mixture of 88% formic acid: 30% peroxide oxygen) was added to samples, which were then oxidized at 4 ºC overnight. Subsequently, performic acid was eliminated by adding sodium meta-bisulfite and intermittently vortexed for at least 2 hr prior to starting hydrolysis with 6M HCL for a 24 hr period (Sedgwick et al., 1991; method 994.12; AOAC, 2005; Cowieson and Ravindran, 2008b). Trp and Pro were not determined in the current study.

The apparent ileal digestibility of CP and AA of the diets was calculated according to the following formula (Olukosi et al., 2007):

Nutrient digestibility (%) =

$\{1-[(AIA_{feed}/AIA_{digesta}) \times (Nutrient_{digesta}/Nutrient_{feed})]\} \times 100$

3.2.4 Statistical analyses

The experiment was conducted as a completely randomized design and ileal digestibility of dietary CP and AA were analyzed by ANOVA (Kuehl, 2000) using the GLM procedure of SAS to examine the effects of dietary treatments (SAS Institute, 2002; Olukosi et al., 2007b). These analyses were done for each corn sample within each dietary phase (i.e., starter, grower, and finisher) with 8 observations (replicates) for each dietary treatment. The cage was the experimental unit and least-squares means were compared using orthogonal contrasts (SAS Institute, 2002, Olukosi et al., 2007b). Differences were considered significant at $P \leq 0.05$.

3.3 Results

Corn 1: Diets had no effects ($P > 0.05$) on ileal digestibility of CP and AA in the starter phase (Tables 3.1 and 3.2). In the grower phase, product X increased digestibility of Arg (by 2.0%) compared to the NC diet. Product XAP increased digestibility of CP (by 3.2%), Arg (by 2.0%), Val (by 3.3%), Ser (by 4.5%), and Ala (by 3.0%). The XG supplementation enhanced digestibility of Arg (by 1.9%) only. However, in the finisher phase, ileal digestibility of CP and some of AA of the NC diet was higher than the PC diet and product X decreased ($P < 0.05$) digestibility of CP, His, Cys, Met, Val, Ile, Leu, Lys, and Ala compared to the NC diet (Tables 3.1 and 3.2).

Corn 2: Dietary treatments had no effect ($P > 0.05$) on digestibility of CP and AA in the starter phase (Tables 3.3 and 3.4). In the grower phase, product X increased ($P < 0.05$) digestibility of CP and all AA except for Met compared to the NC diet. Inclusion of XAP enhanced ($P < 0.05$) ileal digestibility of CP and all AA with the exception of His and Met. Diet supplementation with XG increased ($P < 0.05$) digestibility of CP and all AA except Cys and Met. In the finisher phase, product X increased ($P < 0.05$) digestibility of CP, Thr, Arg, Ile, Lys, Gly, and Ala compared to the NC diet. The XAP treatment followed a similar pattern as product X except that it also increased ($P < 0.05$) digestibility of Val and Phe, but not Ala. However, inclusion of product XG increased ($P < 0.05$) digestibility of Ser only.

Corn 3: There was no effect of diets ($P > 0.05$) on digestibility of CP and AA in the starter and grower phases (Tables 3.5 and 3.6). In the finisher phase, the X diet increased ($P < 0.05$) digestibility of Cys only compared to the NC diet.

However, the XG diet enhanced ($P < 0.05$) ileal digestibility of CP and all AA except for His, Cys, and Met.

3.4 Discussion

There were no effects of enzyme products on ileal digestibility of CP in the starter phase of the present study. The lack of effects of enzyme treatments on CP digestibility in this phase is in agreement with the study of Mahagna et al. (1995) who also did not observe any positive effects of addition of exogenous amylase and protease on CP digestibility of sorghum-soy diets in 14 d old broiler chickens. In the grower phase of the present study, each of the enzyme products increased ileal CP digestibility in birds fed Corn 2 diets, however, only XAP supplementation increased digestibility of CP in the Corn 1 diet. Wyatt et al. (1999) also reported that addition of a mixture of enzymes containing xylanase, amylase, and protease increased ileal digestibility of CP in corn-soy diets in 28-d-old broilers. In another study by Rutherfurd et al. (2007), addition of an enzyme mixture of xylanase, amylase, and β-glucanase in a corn-soy diet significantly increased ileal nitrogen digestibility in 29 d old broiler chickens.

The response patterns observed in the present study can suggest that in some instances, exogenous enzymes may not exert positive effects at early stages of life, and there could still be a possibility for further enhancements in nutrient digestibility at later stages (e.g., grower phase) as well (Gracia et al., 2003).

Ileal digestibility of AA also followed a pattern similar to CP digestibility. There were no effects of enzyme supplementation on the digestibility of AA in the starter phase of the present study. Cowieson et al. (2010) also reported that

xylanase and glucanase (added separately into diets) had no effects on ileal digestibility of AA in the starter phase in birds fed corn-soy diets.

Effects of enzyme supplementation on ileal digestibility of AA were mainly observed in the grower phase of the present study, although the extent of enhancement in digestibility was not the same for all AA. Previous studies with corn-soy diets (Zanella et al., 1999; Cowieson et al., 2006b; Rutherfurd et al., 2007; Cowieson and Ravindran 2008 a, b) have also demonstrated that increases in digestibility of AA were not equal for all AA. In the present study, enzyme products had no effects on Met digestibility which is in accordance with other studies (Zanella et al., 1999; Cowieson et al. 2006b; Rutherfurd et al., 2007; Cowieson and Ravindran, 2008b) which also reported the smallest increases in digestibility of Met compared to other AA following exogenous enzyme supplementation. Rutherfurd et al. (2007) showed that addition of an enzyme product composed of xylanase, amylase, and β-glucanase increased digestibility of Met from 89 to 92%. It appears that, regardless of enzyme supplementation, the ileal digestibility of Met in corn-soy rations is inherently high (Rutherfurd et al., 2007; Cowieson, 2010).

Inherent digestibility of AA should always be considered when evaluating the effects of exogenous enzymes on the ileal digestibility of AA. For instance, Met is highly digestible and exogenous enzymes may not be able to elicit a great response as compared to other AA (Cowieson and Bedford, 2009; Cowieson, 2010; Cowieson et al., 2010).

A variety of mechanisms of action of exogenous enzyme products in corn-soy diets have been proposed. Enhancing digestion and absorption of dietary AA in the digestive tract is one of these suggested mechanisms (Cowieson and Ravindran, 2008 a, b). Carbohydrase enzymes can hydrolyze or solubilize carbohydrate-protein complexes, facilitating the proteolysis of protein constituents of complex structures, leading to an increase in the ileal protein digestibility (Marsman et al., 1997; Meng et al., 2005). Meng and Slominski (2005) suggested that the effects of cell wall-degrading enzymes in increasing ileal digestibility of protein in corn-soy diets may be due to changes in the polysaccharides components of cell wall in soybean, leading to the liberation of protein molecules. This liberation can increase the exposure of dietary proteins to endogenous enzymes present in the digestive tract and increase ileal digestibility of both AA and energy of corn-soy diets. Information on energy digestibility is reported in Chapter 2. Protease enzyme contributes to the hydrolysis of large protein molecules into more absorbable peptides and amino acids, enhancing the overall digestion and absorption of CP and AA (Sheppy, 2001). Product XAP used in the present study contained protease activity and the effects of this product on increasing digestibility of CP and some of the AA might be attributed, to some extent, to this enzyme activity.

Decreasing endogenous losses of nitrogen and AA in the digestive tract of the animal has also been cited as an important possible mechanism contributing to enzyme-induced increases in the ileal digestibility of AA (Hew et al. 1998; Wyatt et al., 1999; Zanella et al., 1999; Hong et al., 2002; Cowieson et al., 2006 a,b;

Cowieson and Ravindran, 2008 a,b). Reductions in endogenous losses are often associated with increases in the digestibility of some of AA that are present in endogenous secretions (Cowieson et al., 2006b; Cowieson and Ravindran, 2008b). Exogenous enzymes used in the present study might have alleviated the negative effects of anti-nutrients in corn-soy diets, resulting in a decrease in the endogenous secretions, which in turn increased ileal digestibility of AA such as Thr, Gly, and Val that are present in endogenous secretions (Hew et al., 1998; Cowieson et al., 2006b; Cowieson and Ravindran, 2007). Pirgozliev et al. (2011) reported a negative correlation between Thr digestibility and endogenous losses of sialic acid (as indicator of endogenous secretion of mucin). It was suggested that increases in the ileal digestibility of Thr could be, to some extent, due to reduction in mucin production resulting from exogenous enzyme supplementation.

Effects of enzyme products observed in the present study were dependent on corn source, enzyme product profile, and stage of the production of the birds. The greatest positive responses to enzyme product addition were mainly found in the grower phase and mostly observed in birds fed corn 2 diets. As stated earlier, these 3 corn samples had similar analyzed nutrient contents, however, enzyme supplementation resulted in different effects on the ileal digestibility of CP and AA in these samples. One explanation for the inconsistency or lack of effects of enzyme products in birds fed Corn 1 and Corn 3 diets might be that there was, in some cases, no difference in digestibility of CP and AA between the PC and NC diets. In some instances, digestibility of the NC diets was even higher than the PC diets. This could be due to high digestibility of Corn 1 and Corn 3 diets compared

to Corn 2 as enzyme responses, to a large extent, are dependent on the nutritional quality of the diet (Olukosi et al., 2007; Cowieson and Bedford, 2009). Cowieson et al. (2010) reported a strong relationship ($R^2 = 0.69$) between digestibility of NC diets and the extent of responses to enzyme supplementation in these diets.

In conclusion, some of the enzyme treatments used in the present study increased ileal digestibility of CP and AA, although these effects were not consistent and varied across corn sources, enzyme products, and dietary phases. Enzyme products elicited the most consistent positive responses in birds fed Corn 2 diets mainly in the grower phase. Based on these results, it can be concluded that factors related to diet (e.g., availability of substrates in Corn 2 diets) and birds (e.g., enzymatic activities in the digestive tract of the birds in the grower phase of this study) may have limited nutrient digestibility in birds and this limitation may have provided favorable conditions for exogenous enzyme products to specifically work on their substrates, resulting in increased digestibility of CP and AA (Cowieson et al., 2006a).

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Table 3.1 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 1 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	CP	His	Thr	Arg	Cys	Met	Val	Phe	Ile	Leu	Lys
Starter											
PC[2]	63.3	72.4	49.3	78.9	36.8	85.8	59.2	67.2	63.3	67.4	75.4
NC[3]	66.4	73.1	52.0	77.2	42.1	87.5	63.2	69.4	66.4	69.9	75.9
NC+X[4]	65.5	71.6	52.4	77.7	40.7	86.8	63.3	68.8	65.6	69.1	76.0
NC+XAP[5]	69.3	77.9	55.7	79.9	45.7	86.9	66.6	72.7	69.8	72.7	79.6
NC+XG[6]	67.3	74.7	55.0	79.1	44.0	86.6	64.2	70.3	66.9	70.4	76.9
SEM	1.80	1.90	2.71	1.70	4.30	1.14	2.08	1.76	1.91	1.62	1.45
P-value[7]											
PC vs. NC	0.262	0.788	0.506	0.488	0.421	0.343	0.201	0.413	0.280	0.297	0.801
NC vs. NC+X	0.736	0.566	0.920	0.837	0.828	0.675	0.979	0.809	0.775	0.744	0.982
NC vs. NC+XAP	0.266	0.090	0.340	0.262	0.563	0.719	0.252	0.193	0.217	0.238	0.082
NC vs. NC+XG	0.722	0.568	0.441	0.423	0.755	0.607	0.727	0.728	0.848	0.831	0.636
Grower											
PC	82.3	91.5	77.3	89.4	73.4	94.4	83.7	86.0	83.8	86.8	90.3
NC	78.9	88.3	71.7	87.1	66.0	93.1	80.3	82.9	80.9	84.1	88.1
NC+X	80.8	88.9	74.3	88.9	67.3	94.3	82.3	84.6	82.8	85.1	88.8
NC+XAP	81.5	88.8	75.3	88.9	69.0	94.8	83.0	85.2	82.8	85.2	89.2
NC+XG	80.3	88.6	74.0	88.8	66.2	94.0	81.2	83.7	82.6	84.9	88.6
SEM	0.83	1.09	1.28	0.48	1.76	0.655	0.90	0.80	0.71	0.61	0.55
P-values											
PC vs. NC	0.007	0.049	0.004	0.002	0.006	0.150	0.010	0.011	0.007	0.003	0.008
NC vs. NC+X	0.121	0.706	0.153	0.016	0.597	0.186	0.127	0.142	0.078	0.234	0.350
NC vs. NC+XAP	0.036	0.748	0.054	0.014	0.241	0.065	0.037	0.058	0.078	0.189	0.153
NC vs. NC+XG	0.265	0.872	0.208	0.020	0.952	0.306	0.477	0.498	0.113	0.355	0.485
Finisher											
PC	80.6	89.1	75.2	88.0	70.0	94.3	81.8	84.8	82.1	85.0	88.2
NC	83.9	90.2	78.6	89.1	78.4	95.4	84.9	86.5	84.9	87.1	90.0
NC+X	81.2	88.2	75.6	87.6	73.2	94.6	82.6	84.4	81.5	84.4	87.9
NC+XAP	83.0	90.0	77.5	89.1	75.0	95.1	83.9	86.0	84.4	86.7	89.3
NC+XG	84.0	90.8	79.0	89.1	76.1	95.6	85.3	87.0	84.8	87.2	90.0
SEM	0.77	0.69	1.07	0.66	1.76	0.29	0.78	0.75	0.79	0.65	0.61
P-values											
PC vs. NC	0.007	0.309	0.035	0.281	0.003	0.016	0.011	0.129	0.023	0.039	0.050
NC vs. NC+X	0.019	0.045	0.057	0.135	0.047	0.041	0.046	0.052	0.005	0.006	0.022
NC vs. NC+XAP	0.419	0.870	0.469	0.999	0.192	0.436	0.374	0.627	0.627	0.661	0.414
NC vs. NC+XG	0.933	0.550	0.788	0.970	0.373	0.754	0.768	0.688	0.946	0.903	0.977

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Table 3.2 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 1 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	Asp	Glu	Ser	Gly	Ala	Tyr
Starter						
PC[2]	61.8	72.8	59.9	59.1	62.9	57.9
NC[3]	64.0	74.2	62.9	61.1	64.8	62.3
NC+X[4]	64.5	74.1	61.9	59.9	65.0	60.1
NC+XAP[5]	67.4	76.7	65.6	64.3	68.5	64.7
NC+XG[6]	66.0	75.8	63.9	64.3	66.5	63.1
SEM	2.12	1.68	2.33	2.37	1.91	1.92
P-value [7]						
PC vs. NC	0.495	0.574	0.395	0.573	0.486	0.126
NC vs. NC+X	0.873	0.979	0.774	0.716	0.957	0.418
NC vs. NC+XAP	0.271	0.300	0.419	0.360	0.185	0.394
NC vs. NC+XG	0.510	0.511	0.775	0.347	0.549	0.793
Grower						
PC	81.4	87.5	81.6	81.4	85.8	79.6
NC	77.1	84.7	76.1	77.0	82.0	76.3
NC+X	79.2	86.4	78.5	79.0	84.4	77.4
NC+XAP	79.9	86.9	79.6	79.9	84.5	78.7
NC+XG	78.9	86.4	77.3	78.6	84.2	78.4
SEM	1.19	0.80	1.15	1.14	0.89	1.22
P-values						
PC vs. NC	0.015	0.018	0.002	0.010	0.004	0.057
NC vs. NC+X	0.209	0.145	0.154	0.234	0.062	0.503
NC vs. NC+XAP	0.101	0.057	0.040	0.084	0.050	0.172
NC vs. NC+XG	0.278	0.154	0.455	0.331	0.088	0.214
Finisher						
PC	80.0	88.0	79.4	78.9	85.2	80.4
NC	82.0	89.0	81.7	82.2	87.5	82.4
NC+X	79.9	87.8	79.8	79.4	85.2	79.3
NC+XAP	81.3	88.8	81.5	82.5	86.6	81.0
NC+XG	82.7	89.6	82.2	82.4	87.6	82.6
SEM	1.01	0.62	1.01	1.13	0.74	1.12
P-values						
PC vs. NC	0.199	0.272	0.131	0.054	0.043	0.236
NC vs. NC+X	0.163	0.175	0.203	0.088	0.039	0.064
NC vs. NC+XAP	0.644	0.795	0.900	0.853	0.422	0.386
NC vs. NC+XG	0.630	0.540	0.689	0.920	0.941	0.881

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Table 3.3 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 2 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	CP	His	Thr	Arg	Cys	Met	Val	Phe	Ile	Leu	Lys
Starter											
PC[2]	70.2	78.2	59.5	79.7	53.7	88.0	69.1	75.1	72.6	75.39	81.2
NC[3]	69.8	77.1	60.4	81.3	54.9	88.6	68.6	74.8	71.8	75.03	80.1
NC+X[4]	71.6	80.9	65.4	81.8	54.2	86.3	70.5	75.2	72.4	75.25	80.5
NC+XAP[5]	71.0	78.2	62.9	81.6	51.2	89.2	70.8	74.9	72.9	75.63	80.8
NC+XG[6]	70.9	79.2	62.1	82.5	51.2	89.2	70.3	75.0	73.4	75.23	81.6
SEM	1.48	1.64	2.19	1.15	2.67	1.32	1.75	1.40	1.63	1.47	1.23
P-value [7]											
PC vs. NC	0.879	0.656	0.772	0.356	0.771	0.760	0.840	0.878	0.736	0.867	0.556
NC vs. NC+X	0.397	0.110	0.113	0.746	0.860	0.213	0.447	0.849	0.790	0.915	0.840
NC vs. NC+XAP	0.589	0.652	0.428	0.841	0.333	0.752	0.367	0.959	0.636	0.772	0.692
NC vs. NC+XG	0.591	0.367	0.587	0.479	0.329	0.741	0.499	0.899	0.497	0.926	0.409
Grower											
PC	79.6	86.9	71.7	86.3	66.3	93.3	79.2	82.5	80.6	83.7	88.1
NC	74.3	82.4	66.0	81.5	61.8	92.7	74.5	77.5	74.7	79.2	83.8
NC+X	79.1	86.1	70.6	85.6	67.2	91.1	79.1	82.0	79.1	82.7	86.6
NC+XAP	79.1	82.0	70.5	85.7	68.5	93.7	78.8	81.5	79.3	82.8	86.8
NC+XG	79.2	85.7	70.5	86.4	66.1	94.1	79.9	82.9	80.6	84.0	87.0
SEM	0.66	1.04	1.17	0.66	1.53	0.59	0.73	0.67	0.90	0.66	0.73
P-values											
PC vs. NC	<0.001	0.004	0.001	<0.001	0.039	0.449	<0.001	<0.001	<0.001	<0.001	0.002
NC vs. NC+X	<0.001	0.018	0.007	<0.001	0.015	0.068	<0.001	<0.001	0.001	0.001	0.008
NC vs. NC+XAP	<0.001	0.785	0.010	0.001	0.004	0.227	0.002	0.002	0.001	0.001	0.007
NC vs. NC+XG	<0.001	0.030	0.009	<0.001	0.057	0.095	<0.001	<0.001	<0.001	<0.001	0.003
Finisher											
PC	83.3	86.1	79.4	89.4	76.2	94.6	83.9	86.3	85.1	86.8	89.7
NC	81.0	86.2	77.3	87.2	73.8	94.7	82.6	84.7	83.4	85.9	89.0
NC+X	83.2	87.6	80.4	89.0	76.9	95.2	84.6	86.2	85.4	87.4	90.4
NC+XAP	83.4	88.4	81.0	88.9	77.0	95.5	85.1	86.7	85.5	87.5	90.4
NC+XG	80.6	84.8	75.2	86.9	72.8	94.5	81.8	84.2	82.6	85.0	88.6
SEM	0.61	1.30	0.97	0.56	1.61	0.38	0.73	0.68	0.61	0.60	0.46
P-values											
PC vs. NC	0.009	0.978	0.133	0.011	0.299	0.819	0.212	0.110	0.058	0.273	0.298
NC vs. NC+X	0.015	0.426	0.033	0.035	0.191	0.329	0.055	0.136	0.026	0.085	0.044
NC vs. NC+XAP	0.006	0.241	0.011	0.039	0.177	0.156	0.018	0.046	0.021	0.063	0.044
NC vs. NC+XG	0.657	0.466	0.118	0.636	0.647	0.715	0.465	0.608	0.338	0.319	0.518

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Table 3.4 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 2 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	Asp	Glu	Ser	Gly	Ala	Tyr
Starter						
PC[2]	69.3	78.7	68.8	65.0	70.3	65.6
NC[3]	70.3	78.8	70.1	65.8	70.8	67.0
NC+X[4]	73.9	81.4	72.9	69.6	72.9	68.8
NC+XAP[5]	72.8	80.7	71.4	67.2	71.8	68.2
NC+XG[6]	71.5	79.6	69.9	68.1	72.3	67.2
SEM	1.64	1.25	1.61	1.99	1.59	1.98
P-value [7]						
PC vs. NC	0.656	0.957	0.577	0.776	0.815	0.621
NC vs. NC+X	0.133	0.150	0.212	0.179	0.356	0.514
NC vs. NC+XAP	0.290	0.276	0.552	0.637	0.670	0.654
NC vs. NC+XG	0.630	0.643	0.948	0.427	0.507	0.936
Grower						
PC	78.6	86.2	76.4	77.0	82.1	74.6
NC	70.2	80.7	70.8	71.5	77.8	69.0
NC+X	77.0	85.5	76.8	75.7	81.5	76.9
NC+XAP	75.9	84.6	76.5	75.8	81.6	75.6
NC+XG	76.8	85.6	75.6	76.6	82.4	75.3
SEM	1.09	0.79	1.03	1.11	0.845	1.34
P-values						
PC vs. NC	<0.001	<0.001	0.004	0.001	0.001	0.005
NC vs. NC+X	<0.001	0.001	0.002	0.010	0.003	0.002
NC vs. NC+XAP	0.001	0.002	0.005	0.011	0.004	0.002
NC vs. NC+XG	0.001	<0.001	0.002	0.003	0.001	0.002
Finisher						
PC	84.7	90.4	83.0	81.9	86.0	83.4
NC	81.9	89.2	80.5	79.5	85.2	81.3
NC+X	83.7	90.1	81.9	82.4	87.2	83.6
NC+XAP	83.9	90.1	81.6	83.4	86.9	83.4
NC+XG	79.9	87.5	77.3	77.4	84.6	80.8
SEM	1.02	0.67	1.10	0.97	0.63	0.95
P-values						
PC vs. NC	0.057	0.227	0.119	0.084	0.375	0.125
NC vs. NC+X	0.213	0.396	0.380	0.038	0.027	0.093
NC vs. NC+XAP	0.179	0.355	0.476	0.007	0.061	0.125
NC vs. NC+XG	0.181	0.073	0.046	0.151	0.514	0.712

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Table 3.5 Effects of dietary treatments on apparent ileal CP and essential AA digestibility in birds fed Corn 3 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	CP	His	Thr	Arg	Cys	Met	Val	Phe	Ile	Leu	Lys
Starter											
PC[2]	66.7	72.4	52.5	77.0	41.4	84.5	63.5	69.2	65.9	68.8	75.5
NC[3]	66.7	71.0	52.6	77.1	42.7	86.3	64.2	70.0	67.1	69.2	77.4
NC+X[4]	64.5	71.1	49.1	74.3	41.4	84.0	61.7	67.3	64.5	66.2	75.1
NC+XAP[5]	69.0	73.7	57.0	78.3	45.1	85.8	65.6	71.1	69.3	71.5	78.6
NC+XG[6]	67.3	75.4	55.9	77.0	44.0	84.6	65.7	70.3	68.7	71.2	77.9
SEM	1.80	1.94	2.52	1.53	3.91	1.68	2.01	1.80	2.03	1.90	1.56
P-value [7]											
PC vs. NC	0.998	0.610	0.975	0.950	0.824	0.449	0.817	0.759	0.675	0.861	0.390
NC vs. NC+X	0.386	0.964	0.338	0.197	0.821	0.346	0.394	0.296	0.361	0.261	0.303
NC vs. NC+XAP	0.374	0.319	0.223	0.590	0.659	0.823	0.609	0.654	0.456	0.401	0.599
NC vs. NC+XG	0.808	0.114	0.354	0.940	0.816	0.487	0.606	0.903	0.583	0.473	0.805
Grower											
PC	79.7	85.4	72.0	86.6	71.4	93.9	80.2	83.7	80.9	83.6	87.8
NC	78.2	85.9	69.8	85.2	66.9	91.7	77.5	81.4	78.5	81.9	86.7
NC+X	79.2	87.6	71.7	86.6	68.7	93.0	78.8	81.9	79.0	82.2	86.8
NC+XAP	77.3	84.7	67.7	85.5	66.5	94.0	76.4	81.1	77.4	80.7	85.1
NC+XG	76.8	84.1	67.4	84.8	66.7	92.3	76.7	80.8	76.7	80.7	85.6
SEM	1.01	1.20	1.65	0.69	2.12	1.32	1.11	1.07	1.12	1.05	0.73
P-values											
PC vs. NC	0.285	0.761	0.356	0.157	0.141	0.233	0.083	0.136	0.136	0.239	0.262
NC vs. NC+X	0.500	0.326	0.440	0.161	0.570	0.492	0.404	0.762	0.741	0.834	0.917
NC vs. NC+XAP	0.518	0.501	0.370	0.747	0.890	0.218	0.497	0.849	0.502	0.435	0.137
NC vs. NC+XG	0.310	0.306	0.296	0.717	0.933	0.729	0.614	0.691	0.252	0.420	0.316
Finisher											
PC	81.9	85.4	76.9	88.5	71.8	94.3	82.3	85.2	83.2	85.1	89.1
NC	79.3	85.2	74.0	86.1	69.9	94.6	79.3	82.1	79.9	82.3	86.6
NC+X	81.4	83.4	75.8	86.6	75.2	95.0	81.3	83.6	81.9	84.2	88.1
NC+XAP	80.7	84.6	75.8	87.0	74.3	94.7	80.8	83.2	81.6	83.4	87.8
NC+XG	82.5	86.9	77.2	88.1	73.2	94.7	82.6	84.9	83.0	85.2	88.9
SEM	0.80	1.07	1.02	0.55	1.54	0.44	0.83	0.79	0.90	0.87	0.59
P-values											
PC vs. NC	0.027	0.922	0.046	0.004	0.377	0.676	0.016	0.008	0.013	0.030	0.005
NC vs. NC+X	0.079	0.227	0.218	0.501	0.020	0.462	0.094	0.205	0.117	0.131	0.072
NC vs. NC+XAP	0.220	0.670	0.200	0.271	0.052	0.796	0.211	0.352	0.198	0.376	0.166
NC vs. NC+XG	0.008	0.266	0.032	0.012	0.133	0.811	0.008	0.017	0.020	0.024	0.010

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Table 3.6 Effects of dietary treatments on apparent ileal CP and non-essential AA digestibility in birds fed Corn 3 diets in the starter (0-11 d), grower (12-28 d) and finisher (29-39 d) phases (%) - DM basis[1]

Diets	Asp	Glu	Ser	Gly	Ala	Tyr
Starter						
PC[2]	63.0	74.1	62.2	56.0	65.2	63.7
NC[3]	64.0	75.1	63.3	56.5	65.9	64.0
NC+X[4]	62.8	74.1	59.9	53.9	63.1	59.8
NC+XAP[5]	65.2	75.8	64.6	59.7	67.8	65.6
NC+XG[6]	67.5	78.0	64.6	59.5	66.9	63.7
SEM	1.97	1.51	2.14	2.48	2.00	1.77
P-value [7]						
PC vs. NC	0.722	0.641	0.714	0.888	0.823	0.921
NC vs. NC+X	0.669	0.662	0.269	0.464	0.325	0.105
NC vs. NC+XAP	0.659	0.727	0.675	0.358	0.495	0.523
NC vs. NC+XG	0.207	0.178	0.675	0.395	0.715	0.901
Grower						
PC	78.1	86.0	79.4	78.3	82.5	77.5
NC	76.6	85.1	77.0	75.4	81.4	75.0
NC+X	77.7	85.4	78.2	77.3	82.4	76.0
NC+XAP	75.2	84.3	75.7	73.9	80.1	74.8
NC+XG	73.9	83.2	75.4	73.2	79.6	74.7
SEM	1.28	0.90	1.20	1.45	1.14	1.27
P-values						
PC vs. NC	0.416	0.451	0.172	0.165	0.488	0.161
NC vs. NC+X	0.547	0.773	0.505	0.377	0.535	0.560
NC vs. NC+XAP	0.452	0.556	0.416	0.466	0.406	0.933
NC vs. NC+XG	0.145	0.155	0.326	0.294	0.252	0.889
Finisher						
PC	81.3	88.1	79.4	79.5	85.5	81.7
NC	78.7	87.1	76.8	76.9	83.4	79.1
NC+X	80.3	88.1	77.9	79.3	84.9	80.4
NC+XAP	80.8	88.2	78.5	78.8	84.7	79.0
NC+XG	81.8	89.0	80.2	80.0	86.4	82.0
SEM	0.96	0.63	0.95	1.02	0.71	0.88
P-values						
PC vs. NC	0.065	0.267	0.056	0.076	0.041	0.051
NC vs. NC+X	0.256	0.279	0.388	0.099	0.137	0.307
NC vs. NC+XAP	0.142	0.249	0.205	0.201	0.203	0.936
NC vs. NC+XG	0.033	0.041	0.016	0.038	0.004	0.030

[1]All means are average of 8 cages per treatment
[2]Positive control
[3]Negative control
[4]Product X= Xylanase
[5]Product XAP = Xylanase, amylase, and protease
[6]Product XG = Xylanase + β-glucanase
[7]Significance level ($P \leq 0.05$)

Chapter 4

Prediction of energetic value of wheat and triticale in broiler chicks: A chick bioassay and an *in vitro* digestibility technique[1]

4.1 Introduction

Wheat is a major feed ingredient commonly incorporated into poultry rations in Europe, Canada, and Australia (Scott et al., 1998a; Pirgozliev et al., 2001). According to the United States Department of Agriculture (2011), estimated global production of wheat was 651.58 million tonnes for the 2010/2011 crop year. Wheat can generally be included at up to 55% in broiler diets thereby providing 60 to 65% and 35 to 40% of dietary apparent metabolizable energy (**AME**) and protein, respectively (Gutierrez del Alamo et al., 2009a). Although wheat provides protein, its energy contribution to the diet is the major consideration from economic standpoint (Pirgozliev et al., 2001).

Wheat is as an ingredient with wide variations in nutrient content (Hughes and Choct, 1999). The AME values can vary substantially among wheat samples (Scott et al., 1998a). Rogel et al. (1987) observed substantial variations in AME values among 38 varieties of wheat, with values ranging from 2,484 to 3,511 kcal/kg.

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[1]A version of this chapter has been submitted for publication in Animal Feed Science and Technology (Yegani, M., M. L. Swift, R. T. Zijlstra, and D. R. Korver. 2012. Prediction of energetic value of wheat and triticale in broiler chicks: A chick bioassay and an *in vitro* digestibility technique (*Under Review*).

Access to information on the nutritive value of specific batches of feedstuffs used at the commercial level is limited. Thus, using rapid, inexpensive, and accurate methods is of critical importance to help the feed industry to make necessary modifications in commercial diet composition to ensure consistent dietary AME (Hughes and Choct, 1999). Energy is the most expensive constituent of diets and the ability to predict the energy content of wheat samples can increase the accuracy of diet formulation (Regmi et al., 2009).

Measuring physical characteristics (e.g., 1,000 kernel weight) is a routine practice in the feed industry (Scott et al., 1999), but this approach does not accurately predict the feeding value of wheat samples (Zijlstra et al., 1999; McCracken et al., 2001). Chemical analyses of feed ingredient samples are time-demanding and expensive (Hughes and Choct, 1999), although these provide more information about the feeding value of a given feedstuff. However, these analyses do not directly predict the extent of nutrient availability and use by the animals (van Barneveld, 1999). *In vivo* digestibility trials (i.e., bioassays) can fill in the gap by measuring variations in energy availability of cereal grains for broiler chickens (Scott, 1996), but time and costs are two major factors limiting their application (Zijlstra et al., 2010).

The above-mentioned disadvantages necessitate the use of other feed quality evaluation approaches such as *in vitro* digestibility techniques and near infrared reflectance spectroscopy (NIRS). The NIRS can provide results in a “real-time” manner (Leeson, 1997; Zijlstra, 2006). *In vitro* digestibility techniques can, by simulating the digestive tract conditions of the host animal, predict energy content

of cereal samples. However, these techniques need to be validated by the data obtained from the bioassays. Validated *in vitro* techniques can play an important role in providing data for NIRS calibrations (Zijlstra et al., 2010).

The energetic value of cereal grains including wheat can vary considerably and *in vitro* digestibility methods can predict these variations in swine (Regmi et al., 2009; Zijlstra et al., 2010). Few have attempted to develop *in vitro* energy digestibility techniques in poultry (Clunies et al., 1984; Valdes and Leeson, 1992; Losada et al., 2009), and two main limitations exist. First, these *in vitro* methods were mostly validated with *in vivo* data from adult roosters not broiler chickens. Second, a wide range of ingredients or diets of various compositions were used. Thus, an *in vitro* method must be developed that is validated in broiler chickens to specifically predict the AME of wheat samples.

The hypothesis was that *in vitro* digestibility techniques could predict variation in AME values of wheat samples for broiler chicks. The objectives were to determine the nutritive value of 8 test samples including six wheat and two triticale in a modified broiler chick bioassay and a two-step *in vitro* digestibility technique was evaluated to predict *in vivo* AME of these cereal samples for broiler chicks.

4.2 Materials and methods

4.2.1 Test samples

Six samples of wheat of different classes were obtained from various farms in western Canada. These classes were: Canada Prairie Spring Red (**CPSR**), Canada Western Red Spring (**CWRS**), Canada Western Soft White Spring

(**CWSWS**), Canada Western Hard White Spring (**CWHWS**), Canada Western Amber Durum (**CWAD**), and Canada Western Red Winter (**CWRW**). In addition, two samples of spring triticale (Triticale 2005 and Triticale 2006; varieties unknown) were evaluated.

Test weight and 1,000 kernel weight of samples were measured at the Field Crop Development Centre of Alberta Agriculture and Rural Development (Lacombe, AB, Canada) according to the guidelines of the Canadian Grain Commission (Winnipeg, MB, Canada).

4.2.2 Chick bioassay

This study was approved by the Animal Care and Use Committee: Livestock of the University of Alberta and met guidelines of the Canadian Council on Animal Care (CCAC, 1993). A total of 768 one-d-old male broiler chicks (Ross 308, Aviagen, Huntsville, AL) were randomly assigned into groups of 12 chicks to 64 Specht pullet cages (53 × 59 × 44 cm, Specht Canada Inc., Stony Plain, AB, Canada). The room temperature was initially set at 34ºC, and was accordingly decreased to the end of the experiment at 13 d of age. The lighting program was 23 h light: 1 h dark per day and the birds had unrestricted access to feed and water throughout the feeding trial.

Birds were fed a nutritionally complete, commercial-type wheat-based starter diet from 0 to 7 d of age, and experimental diets were fed from 8 to 13 d of age. Eight test samples varying in nutrient contents were mixed into 8 experimental diets. Each sample of wheat or triticale was included at 80% of the diet (Scott et al., 1998b) and the remaining 20% was a mixture of other ingredients including

soybean meal and fish meal (Table 4.1). Ingredient composition of the mixture was different from what Scott et al. (1998b) used in their study. Celite (Celite Corporation, World Minerals Inc., Lompoc, CA) was included at 1% in all experimental diets as an indigestible ash marker for determination of apparent ileal and total tract digestibility of nutrients and energy. The experimental diets were fed to the birds in a mash form.

Each experimental diet was fed to 8 cages of 12 chicks (96 chicks per diet) from day 7 to 13 of the experiment. Body weight (**BW**) and feed intake were recorded and feed conversion ratio (**FCR**) was calculated for each cage. Cage mortality (number and weight of dead birds within each cage) was recorded daily throughout the experiment and FCR was corrected accordingly at the time of calculating growth performance variables. At 13 d of age, all birds in each cage were euthanized by cervical dislocation and contents of the ileum (from Meckel's diverticulum to the ileo-cecal junction) were collected in plastic bags, pooled within each cage, and frozen immediately at -20ºC. Pooled digesta samples were subsequently freeze-dried and ground for laboratory analyses (Garcia et al., 2007). Excreta were collected from a tray placed below each cage for a 48 h period from d 11 to d 13. Excreta samples were prepared for chemical analyses as described for the digesta samples.

4.2.3 Chemical analyses

Dry matter (**DM**) contents of test samples, experimental diets, ileal digesta, and excreta samples were determined (method 934.01; AOAC, 2005). Acid insoluble ash (**AIA**) contents of the samples were also determined (McCarthy et

al., 1974). Gross energy (**GE**) of all samples was measured by bomb calorimetry using an adiabatic calorimeter (IKA® Werke GmbH & Co. KG, Staufen, Germany) standardized with benzoic acid (Nortey et al., 2008). Test samples were analyzed for ash (method 942.05; AOAC, 2005), ether extract (method 920.39; AOAC, 2005), and neutral detergent fiber (NDF, van Soest et al., 1991). Starch determination in test samples, experimental diets, and digesta was done using a total starch assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). This procedure uses a combination of thermostable α-amylase and amyloglucosidase. Nitrogen content of samples was determined by combustion with an automatic nitrogen analyzer (Leco TruSpec CN, Leco Corp., St. Joseph, MI), and then multiplied by 6.25 to calculate CP (CP) content of each sample (method 968.06; AOAC, 2005).

The apparent ileal and total tract digestibility of nutrients and energy of the experimental diets were calculated according to the following formulas (Olukosi et al., 2007):

Nutrient digestibility =

$$1-[(AIA_{feed} / AIA_{digesta\ or\ excreta}) \times (Nutrient_{digesta\ or\ excreta} / Nutrient_{feed})]$$

Dietary ileal digestible energy (**IDE**) or AME (kcal/kg) =

$$GE_{diet} - [GE_{digesta\ or\ excreta} \times (AIA_{diet} / AIA_{digesta\ or\ excreta})]$$

Determined dietary AME values were then used to calculate AME of test samples as described by Scott et al. (1998b). Based on the values reported in the NRC (1994), the total energy contribution of the ingredients that made 20% of the diet was 555.9 kcal/kg.

AME of the test sample (kcal/kg) =

$$(\text{AME of the experimental diet} - 555.9) \times 100/80$$

Dietary IDE is a measure of the amount of energy absorbed by the bird up to the ileum, minimizing the confounding effects of hindgut microbiota on energy measurement using excreta samples (Olukosi et al., 2007).

4.2.4 In vitro digestibility technique

A two-step *in vitro* digestibility technique was used to predict *in vivo* AME of the 8 test samples for broiler chicks. This technique was based on a combination of previously described *in vitro* methods (Boisen and Fernandez 1995, 1997; Hervera et al., 2007; Noblet and Jaguelin-Peyraud, 2007; Regmi et al., 2008, 2009; Losada et al., 2009, 2010). Different incubation times and enzyme concentrations were tested as part of efforts to determine the consistency of the *in vitro* digestibility data. The following *in vitro* conditions were associated with the most consistent *in vitro* digestibility results.

Test samples (500 ± 10 mg, 1 mm particle size) were weighed into 125 ml *in vitro* flasks. Three glass beads were added to each flask together with 25 ml of 0.1 M phosphate buffer. Subsequently, 10 ml of 0.2 M HCl was added to reduce pH from 6 to 2. Flasks were then swirled to ensure that samples were mixed with the solution. Then, 1 ml of porcine pepsin (25 mg/ml, P-7000, Sigma-Aldrich) was added. Chloramphenicol (0.5 ml) was added into the flasks to reduce or minimize the effects that bacterial fermentation may exert during the incubation phases. Flasks were again swirled and then placed into a shaking waterbath (100

rpm, 41ºC) for 2 hr. This step was intended to simulate the gastric phase of digestion.

After the first phase, flasks were removed from the waterbath and 10 ml of 0.2 M phosphate buffer and 5 ml of 0.6 M NaOH were added into each flask to raise pH to 6.8. After flasks were swirled, 1 ml of porcine pancreatin containing amylase, lipase, and protease (100 mg/ml, P-1750, Sigma-Aldrich) was added. Flasks were swirled and then incubated in the shaking waterbath for 4 hr under the same speed and temperature as the gastric digestion phase. This step was intended to simulate the post-gastric phase of digestion.

After the second incubation phase, 5 ml of 20% sulfosalicylic acid was added into the flasks. This addition is essential for the termination of enzymatic reactions in the flasks and allows the precipitation of undigested soluble proteins. To achieve this goal, flasks were left for 30 minutes at room temperature. Then, flask contents were filtered using Whatman no. 54 filter papers (Whatman Inc., Florham Park, NJ). Filter papers were dried overnight at 80ºC before being used for the filtration. The *in vitro* residues collected in filter papers were also dried overnight at 80ºC.

The GE of oven-dried *in vitro* residues was measured by calorimetry as described previously to calculate *in vitro* digestibility of GE. *In vitro* digestibility of GE was calculated by the difference between GE of test sample and GE of the *in vitro* residue of the same sample. Then, the *in vitro* digestibility of GE was used to calculate the *in vitro* AME of test samples according to the following formula adapted from Hervera et al. (2007) and Regmi et al. (2009):

In vitro AME of test sample (kg/kg) =

In vitro digestibility of GE of test sample × GE of test sample

4.2.5 Statistical analyses

Growth performance and apparent ileal and total tract digestibility of nutrients and energy were analyzed using the GLM procedure of SAS (SAS Institute, 2002) using cage as the experimental unit. Means were separated using the Student-Newman-Keuls (SNK) test (Kuehl, 2000). Differences were considered significant at $P < 0.05$.

The regression procedure of SAS (SAS Institute, 2002) was used to investigate the relationship between variables including the *in vitro* and *in vivo* AME. Coefficient of determination (R^2) and standard error of prediction (**SEP**) were used to determine the quality of prediction of *in vivo* AME (Regmi et al., 2009). The SEP was calculated according to the following equation (Owens et al., 2009; Regmi et al., 2009):

$$\text{SEP} = \sqrt{\frac{\sum(Y - Y')^2}{N}}$$

where Y is the *in vivo* AME determined in the chick bioassay, Y′ is the predicted *in vivo* AME value based on the *in vitro* data, and N is the number of cereal samples tested in the *in vitro* digestibility technique.

Variations in different characteristics of wheat samples can be classified into 3 groups (Jha et al., 2011): 1) wide variation, CV > 10%; 2) medium variation, CV from 5 to 10%, and 3) small variation, CV < 5%. This classification

will help to explain the results of the present study in the context of variations in nutrient availability among 8 test samples.

4.3 Results

4.3.1 Physico-chemical characteristics of test samples

The 1,000 kernel weight varied widely (CV= 23.1%) among test samples and ranged from 26 g for Triticale 2006 sample to 52 g for CWAD wheat sample. The CV for test weight (kg/hL) was medium (CV=10.0%) and test weight ranged from 59.5 kg/hL for Triticale 2006 sample to 83.0 kg/hL for CWAD wheat sample (Table 4.2).

The CV for DM (CV= 0.3%), GE (CV=1.4%), and starch (CV= 4.0%) was small (Table 4.2). However, CP (CV= 17.7%), ash (CV= 12.9%), ether extract (CV= 12.6%), and NDF (CV= 7.1%) contents varied widely. The CP content varied from 12.1% for CWSWS sample to 19.9% for CWAD sample. Ether extract content ranged from 1.2% in Triticale 2006 sample to 1.8% in CWSWS sample. CWAD and CPSR samples had the lowest (10.6%) and CWSWS and Triticale 2006 samples had the highest (12.6%) NDF contents.

4.3.2 Growth performance variables

Production performance variables did not differ among bird groups at 7 d of age, prior to feeding the experimental diets (data not shown). Feeding experimental diets from 8 to 13 d of age had a significant effect on BW gain and FCR, but there was no effect on feed intake (Table 4.4). Birds fed CWSWS and CWAD-based diets had the lowest and highest BW gain, respectively.

Accordingly, these groups of birds had the lowest (CWAD) and highest (CWSWS) FCR as well, although there was no effect on feed intake.

4.3.3 In vivo digestibility

The CPSR- and CWAD-based diets had the lowest (60.1%) and highest (70.8%) apparent ileal DM digestibility coefficient, respectively (Table 4.5). There were also significant differences in apparent total tract digestibility of DM, which ranged from 65.4% for Triticale 2005 to 73.3% for CWAD. Birds fed with CPSR- and Triticale 2006-based diets had the lowest (83.6%) and highest (93.7%) apparent ileal starch digestibility, respectively.

Birds fed with CPSR- and CWAD-based diets had the lowest (62.9%) and highest (72.3%) apparent ileal digestibility of dietary GE, respectively. Dietary IDE ranged from 2,710 kcal/kg of DM for CWSWS-based diet to 3,196 kcal/kg of DM for CWAD-based diet (Table 4.5). In terms of the total tract GE digestibility of the diets, Triticale 2005 and CWAD-based diets had the lowest (68.5%) and highest (76.3%) digestibility coefficient, respectively. Dietary AME was also different among the grain sources and ranged from 3,005 kcal/kg of DM for CWSWS-based diet to 3,372 kcal/kg of DM for birds fed CWAD-based diet. The AME values of test samples (calculated from dietary AME) varied widely and ranged from 3,061kcal/kg of DM for CWSWS wheat to 3,520 kcal/kg of DM for CWAD wheat.

Birds fed with Triticale 2005- and CWAD-based diets had the lowest (70.2%) and highest (78.8%) apparent ileal digestibility coefficient of CP,

respectively (Table 4.5). A similar pattern was also observed for the total tract digestibility coefficient of CP (50.1and 60.3%, respectively).

Test sample *in vivo* AME and their test weight or 1,000 kernel weight were not related (R^2 = 0.09 and 0.01, respectively; Table 4.6). The AME was not also related to starch (R^2 = 0.35; P = 0.123), ether extract (R^2 = 0.10; P = 0.458) or NDF (R^2 = 0.28; P = 0.176) content of the samples. The AME was related to GE (R^2 = 0.80; P = 0.003), and CP (R^2 = 0.83; P = 0.002). It was also strongly related to BW gain (R^2 = 0.96; P < 0.001) and FCR (R^2 = 0.85; P = 0.001) of the birds from day 7 to 13 of age. However, the relationship between AME and feed intake was moderate (R^2 = 0.45; P = 0.069).

4.3.4 In vitro digestibility

The *in vitro* AME and *in vivo* AME of test samples were strongly related (R^2 = 0.81; P = 0.002). The prediction equation developed from this regression was: *In vivo* AME = - 898.14 +1.1665 × *in vitro* AME, with a SEP of 68.6 kcal/kg. This equation can predict *in vivo* AME of the test samples in a simple and rapid manner. The inclusion of chemical characteristics of test samples into the above-mentioned equation increased the accuracy and precision of *in vivo* AME prediction (Table 4.7). Inclusion of NDF (R^2 = 0.81; SEP = 68.2 kcal/kg; P = 0.015), ether extract (R^2 = 0.83; SEP = 65.7 kcal/kg; P = 0.013), starch (R^2 = 0.90; SEP = 50.5 kcal/kg; P = 0.003), and CP (R^2 = 0.98; SEP = 23.5 kcal/kg; P < 0.001) increased the R^2 and decreased SEP of prediction of *in vivo* AME. Inclusion of two or three chemical characteristics together, in addition to *in vitro* AME, also resulted in increases in R^2 and reduced the SEP. As shown in Tables

4.7 and 4.8, inclusion of NDF, ether extract, starch, and CP content of the samples together resulted in the strongest prediction equation (R^2 = 0.99, SEP =12.5 kcal/kg, P = 0.016).

4.4 Discussion

4.4.1 Physico-chemical characteristics of test samples

Physical and chemical characteristics of the test samples varied widely in the present study, similar to previous reports for physical (Zijlstra et al., 1999; Garnsworthy et al., 2000; Wiseman, 2000; Pirgozliev et al., 2003; Owens et al., 2009; Regmi et al., 2009) and chemical characteristics (Bedford et al., 1998; Austin et al., 1999; Choct et al., 1999; Zijlstra et al., 1999; Pirgozliev et al., 2003; Gutierrez-Alamo et al., 2006; McCracken et al., 2008; Owens et al., 2009). Density of 16 wheat samples from western Canada ranged from 57.8 to 77.6 kg/hL (Zijlstra et al., 1999). The 1,000 kernel weight of 160 wheat samples ranged from 35 to 59 g (Garnsworthy et al., 2000). For 164 wheat samples from different varieties and geographical locations, specific weight and thousand grain weight ranged from 59 to 78 kg/hL and 21.7 to 60.8 g, respectively (Owens et al., 2009).

Physical characteristics and AME values of test samples were not related in the present study similar to previous studies in poultry (Wiseman, 2000) and pigs (Zijlstra et al., 1999). The lack of relationship between wheat physical characteristics and AME indicate that other variables play a more important role with respect to the nutritive value of grain (Dale, 1994). However, there were positive relationships between AME and both GE and CP contents of the samples.

Zijlstra et al. (1999) also reported a positive correlation between digestible energy (**DE**) and CP content of 15 wheat samples in growing pigs. Our findings are in contrast to the negative relationships between nitrogen-corrected AME (**AMEn**) or AME of diet or ingredient and protein content reported previously (Svihus and Gullord, 2002; Gutierrez-Alamo et al., 2006). The reasons for these discrepancies in observations are not clear, but might be related to differences in inclusion rate of wheat and also protein supplementation of the diets among the studies (McCracken and Quintin, 2000).

The AME values were not related with starch, ether extract, or NDF contents of wheat samples in the present study, similar to other studies (Mollah and Annison, 1981; Mollah et al., 1983; Rogel et al., 1987; Austin et al., 1999; Choct et al., 1999). Starch content of wheat samples might not reflect abnormalities in AME content (Mollah et al., 1983) and the lack of relationship between AME and starch content might indicate that starch is not completely digested in birds (Mollah and Annison, 1981). Thus, measuring physical characteristics is not a strong basis for an accurate prediction of AME of cereal samples. However, predictions of energetic value for animals using chemical characteristics of test samples are generally more accurate (Zijlstra et al., 1999).

4.4.2 Growth performance variables

The main challenge in formulating the experimental diets in the present study was related to balancing the diets for CP level as it was required to include test samples at 80% of the diets in the chick bioassay. Thus, the experimental diets used in the present study were not necessarily considered to be practical

diets. This might have played a limiting role in terms of practical relevance of growth performance data of the present study.

Birds fed the CWAD-based diets had the highest and lowest BW gain and FCR, respectively among dietary treatments in the present study. This observation is in agreement with other studies (Bedford et al., 1998). Greater performance of this group of birds may be attributed to the typically lower digesta viscosity and higher AME value of CWAD wheat (Bedford et al., 1998). There were also strong positive relationships between AME of test samples and BW gain and FCR of birds, however, the relationship between AME and feed intake was nearly significant in the present study. Scott et al. (1999) observed a positive correlation between wheat AME and BW, but they found a negative correlation between wheat AME and FCR. Svihus and Gullord (2002) also reported a negative correlation between AME content of wheat diets and feed intake.

4.4.3 In vivo digestibility

Starch is the main energy-providing constituent in wheat and variations in starch digestibility may have an impact on AME values of wheat samples (Wiseman, 2006). Ileal digestibility of dietary starch was different among the treatments in the present study. This is in agreement with Rogel et al. (1987) who also found substantial variation in starch digestibility among 38 wheat cultivars and is in contrast with Gutierrez-Alamo et al. (2006) who did not observe any differences in the ileal starch digestibility of 5 wheat samples collected from 5 different locations in Spain. The average ileal digestibility of dietary starch was 89.67 % in the present study, indicating that about 10% of starch was not digested

in the small intestine. Gutierrez del Alamo et al. (2009a) reported an average distal ileum digestibility of 95.48%, suggesting that only 4% of undigested dietary starch passed the small intestine and entered into the hindgut. The increase in nutrient digestibility with bird age (Batal and Parsons, 2002) might have contributed to differences between our findings with those of Gutierrez del Alamo et al. (2009a), as their measurements were done in 30 d old broiler chickens. However, higher starch digestibility is not necessarily an indication of greater starch digestion rate because digestion rate is related to retention time and also capacity of the digestive tract (Gutierrez del Alamo et al., 2009b). Genetic (i.e., wheat cultivar) and environmental conditions (e.g., location) can contribute to variations in starch digestion rate among wheat samples (Gutierrez del Alamo et al., 2009a, b).

The AME of the test samples and ileal starch digestibility of the diets were not related in the present study. This is in agreement with Gutierrez del Alamo et al. (2008) who also reported no relationship between fecal starch digestibility and AME of wheat samples and is in contrast with other studies (Mollah et al., 1983; Rogel et al., 1987). Mollah et al. (1983) reported a significant correlation (r = 0.91) between AME and total tract starch digestibility of wheat samples. The lack of relationship between starch digestibility and AME value of wheat samples might be attributed to the narrow range in starch content among wheat samples (Zijlstra et al., 1999; Gutierrez del Alamo et al., 2008). The CV of starch content of wheat samples in the present study was 4.0 % which is relatively close to the

5.3 and 1.1 % CV reported by Zijlstra et al. (1999) and Gutierrez del Alamo et al. (2008), respectively.

The *in vivo* AME of the test samples varied widely in the present study (3,061 to 3,520 kcal/kg of DM) which is in line with other studies (Rogel et al., 1987; Bedford et al., 1998; Scott et al. 1998a, b). In addition to differences among wheat samples used in various studies, experimental-related factors (e.g. sources of broiler chicks used and environmental conditions) are also often different and as a result, it is difficult to make direct comparisons among these studies (Pirgozliev et al., 2001; Scott and Pierce, 2001). The AME of wheat samples (nine cultivars grown in 3 locations in two consecutive years in western Canada) in broiler chicks varied from 3,280 to 3,650 kcal/kg; samples of Durum wheat had higher AME values compared to HRS and CPS wheat samples (Scott et al., 1998a). In another study, Scott et al. (1998b) reported that AME of HRS wheat were higher than CPS wheat cultivars. This is an indication that wheat cultivar can play an important role in the variation in AME content and this factor should be considered in diet formulation (Scott et al., 1998a). These observations in AME values are in line with the findings of the present study.

Considering composition of the experimental diets, the major focus of the present study was on variability in energetic values of the test samples. However, digestibility of CP also varied widely among dietary treatments. Bedford et al. (1998) reported a range in ileal CP digestibility of 83 to 88% for 54 wheat samples of nine cultivars from 3 different locations in western Canada. Ileal CP digestibility of wheat-based diets in the present study was between 70.2 to 78.8%

which is in line with 73.5 to 76.9% reported by Gutierrez Alamo et al. (2006). The variations in digestibility of CP among these studies might have been resulted from differences in wheat cultivars and environmental conditions (Bedford et al., 1998), differences in basal diets (Scott et al., 1998a) and also age of birds (Batal and Parsons, 2002).

4.4.4 In vitro digestibility

There was a strong relationship between *in vitro* AME and *in vivo* AME with R^2 = 0.81 and SEP of 68.6 kcal/kg in the present study. Clunies et al. (1984) used a two-step *in vitro* method to predict AMEn of poultry rations which represented a wide range in terms of both ingredients and nutrient levels. They reported a correlation coefficient (r) of 0.93 and the residual standard deviation (RSD) of 145 kcal/kg, but the *in vitro* data were validated with AMEn determined in adult roosters. Valdes and Leeson (1992) also used a two-step *in vitro* digestibility technique to predict AMEn of 71 poultry diets. The R^2 and the standard error of estimate of AMEn of 71 poultry diets were 0.71 and 152 kcal/kg, respectively. The *in vitro* digestibility technique used by Losada et al. (2009) on 6 ingredients (wheat, barley, corn, sorghum, rye, and peas) and 6 cereal by-products developed a prediction equation of AMEn with R^2 of 0.59 and RSD of 275 kcal/kg.

Inclusion of energy-related components such as NDF, starch, ether extract, and CP into the prediction equation increased the R^2 and decreased SEP in the present study. Inclusion of these variables together resulted in the strongest prediction equation of *in vivo* AME (R^2 = 0.99, SEP = 12.5 kcal/kg, P = 0.016).

Inclusion of chemical characteristics and the effects that these additions can have on the accuracy of prediction of the *in vivo* data has been reported previously (Clunies et al., 1984; Noblet and Jaguelin-Peyraud, 2007; Losada et al., 2009). Inclusion of chemical characteristics resulted in development of the *in vivo* AME prediction models with P values less than 0.05 (Losada et al., 2009), although P value of each individual chemical characteristic was not significant in the present study. Inclusion of ether extract into the regression equation was associated with an increase in the accuracy of the prediction of AME of the diets, but the coefficient for ether extract was not significant (Clunies et al., 1984).

Validation of *in vitro* digestibility data with the corresponding data from broiler chick bioassay is important. However, other studies (Clunies et al., 1984; Valdes and Leeson, 1992; Losada et al., 2009) used adult roosters to validate the *in vitro* data. The AME values determined in adult roosters are higher than values in broiler chickens and this difference is a limiting factor for an accurate evaluation of feeding value (e.g., energetic value) of feedstuffs for broiler chickens (Mollah et al., 1983; Svihus and Gullord, 2002). Birds had continuous access to feed in the present study. Continuous feeding is more reflective of commercial poultry farm conditions and as a result, negative effects of starvation (from both physiological and welfare standpoints) are reduced or minimized in this approach compared to when the feed is intubated in adult roosters. Another consideration is that non-starch polysaccharides level in a test diet may have a lesser impact on adult roosters compared to broiler chicks (Scott, 1996; Scott et al., 1998b).

Another important aspect of the *in vitro* assay developed in the present study is that this technique was specifically tested for wheat samples. *In vitro* digestibility techniques reported in previous studies (Clunies et al., 1984; Valdes and Leeson, 1992; Losada et al., 2009) were used for a wide array of diets or ingredients fed to poultry. For instance, Losada et al. (2009) tested 6 ingredients (wheat, barley, corn, sorghum, rye, and peas) and 6 cereal by-products to develop regression equations for prediction of AMEn of these ingredients. Focusing on developing specific *in vitro* digestibility method for each feedstuff is needed because a single *in vitro* method for multiple ingredients might not provide accurate predictions for all ingredients (Valdes and Leeson, 1992; Regmi et al. 2008; Zijlstra et al., 2010). Different ingredients or complete diets can vary in the optimal conditions for *in vitro* digestion assay. These variations may include differences in incubation times, enzyme levels, and the volumes of buffers required to reach a desirable range in pH values throughout the incubation phases. Thus, it is difficult to meet all these requirements when only one *in vitro* digestibility technique is being used for multiple ingredients or diets of different compositions (Valdes and Leeson, 1992).

4.5 Conclusions

Measuring physical characteristics of the test samples did not accurately predict AME of the test samples. Predictions based on chemical characteristics were more accurate in terms of reflecting the bioassay results, but time and costs were associated with these approaches. The *in vitro* digestibility technique increased the accuracy of prediction of *in vivo* AME for broiler chickens. Inclusion of chemical characteristics into prediction equations of *in vivo* responses

resulted in highly accurate predictions of *in vivo* AME of the test samples. Considering that the *in vitro* technique was only tested on 8 samples, testing a large number of wheat samples with wide variations in nutritional quality, as suggested by Regmi et al. (2009), will be of help to create a stronger database for predicting *in vivo* AME of different wheat samples for broilers chickens.

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Table 4.1 Composition of diets fed to broiler chicks

Ingredients (%)	Starter diet (day 0 to7)	Experimental diet (day 8 to13)
Corn	18.00	-
Vegetable fat	3.76	2.00
Fish meal	3.00	3.00
Soybean meal	26.79	8.05
Wheat	43.41	80.00
Calcium carbonate	1.32	1.22
Dicalcium phosphate	1.50	1.55
Salt	0.42	0.35
L-Lysine	0.23	0.83
DL-Methionine	0.23	0.36
L-Threonine	0.05	0.35
Vitamin E 5,000 IU/Kg	0.30	0.30
Celite	-	1.00
Vitamin-mineral premix[1]	0.50	0.50
Choline chloride[2]	0.50	0.50
Calculated nutrient content		
DM (%)	88.8	89.0
CP (%)	23.00	18.00
Metabolizable energy (kcal/kg)	3,079	3,043

[1]Vitamin/mineral premix provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 ICU; vitamin E, 35 IU; menadione, 2.0 mg; D - pantothenic acid, 14 mg; riboflavin, 5.0 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18; iodine, 0.5 mg; manganese, 70 mg; copper, 8.5 mg; zinc, 80 mg; selenium, 0.1 mg; iron, 100 mg
[2]Provided 100 mg of choline per kg of diet

Table 4.2 Chemical composition of 8 test samples (%)-DM basis[1]

Item	CPSR	CWRS	CWSWS	CWHWS	CWAD	CWRW	Triticale 2005	Triticale 2006	CV[2] (%)
Test weight (kg/hL)	80.2	75.8	80.4	78.5	83	76.3	70.3	59.5	10
1,000 kernel weight (g)	42	37	40	32	52	27	38	26	23.1
DM	89.6	89.6	90.3	89.6	89.9	89.8	89.7	89.5	0.3
CP	14.1	19.4	12.1	19.2	19.9	17.6	13.7	17.5	17.7
GE[3] (kcal/kg)	4,378	4,425	4,305	4,486	4,477	4,450	4,354	4,438	1.4
Starch	64.4	59.3	64.8	60.3	60.7	60.0	62.2	57.8	4.0
Ether extract	1.3	1.5	1.8	1.6	1.7	1.6	1.4	1.2	12.6
NDF[4]	10.6	12.0	12.6	11.4	10.6	11.0	12.0	12.6	7.1
Ash	2.1	2.3	2.7	2.6	2.5	2.6	2.3	3.1	12.9

[1]CPSR: Canada Prairie Spring Red; CWRS: Canada Western Red Spring; CWSWS: Canada Western Soft White Spring; CWHWS: Canada Western Hard White Spring; CWAD: Canada Western Amber Durum; CWRW: Canada Western Red Winter
[2]Coefficient of variation
[3]Gross energy
[4]Neutral detergent fiber

Table 4.3 Chemical composition of 8 experimental diets[1] (%)-DM basis

Item	CPSR	CWRS	CWSWS	CWHWS	CWAD	CWRW	Triticale 2005	Triticale 2006
DM	90.5	89.5	90.2	89.5	90.7	89.5	89.6	89.1
GE[2] (kcal/kg)	4,330	4,428	4,264	4,435	4,419	4,456	4,404	4,396
Starch	51.8	46.1	51.7	50.1	46.9	48.1	49.0	44.4
CP	18.5	23.7	18.2	23.3	24.1	21.6	19.1	21.8

[1]CPSR: Canada Prairie Spring Red; CWRS: Canada Western Red Spring; CWSWS: Canada Western Soft White Spring; CWHWS: Canada Western Hard White Spring; CWAD: Canada Western Amber Durum; CWRW: Canada Western Red Winter

[2]Gross energy

Table 4.4 Growth performance variables of birds during day 8 to 13[1,2]

Item	CPSR	CWRS	CWSWS	CWHWS	CWAD	CWRW	Triticale 2005	Triticale 2006	*Pooled SEM*	*P*-value
BW gain (g/bird/d)	18.8[c]	22.2[ab]	17.7[c]	21.9[ab]	24.3[a]	21.6[b]	18.3[c]	19.3[c]	0.72	<0.001
Feed intake (g/bird/d)	32.0	35.0	33.9	35.6	34.7	35.2	33.8	32.5	1.02	0.145
FCR[3] (g feed/g gain)	1.71[bc]	1.60[cd]	1.94[a]	1.63[bcd]	1.43[d]	1.63[bcd]	1.84[ab]	1.69[bc]	0.05	<0.001

[1]CPSR: Canada Prairie Spring Red; CWRS: Canada Western Red Spring; CWSWS: Canada Western Soft White Spring; CWHWS: Canada Western Hard White Spring; CWAD: Canada Western Amber Durum; CWRW: Canada Western Red Winter

[2]All means are average of 8 cages per treatment

[3]Feed conversion ratio

[a-d] Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 4.5 Apparent ileal and total tract digestibility coefficient of nutrients and energy of experimental diets [1,2] (DM basis)

Item	CPSR	CWRS	CWSWS	CWHWS	CWAD	CWRW	Triticale 2005	Triticale 2006	Pooled SEM	*P*- value
Ileal										
DM	60.1[c]	63.2b[c]	61.7[bc]	65.3[b]	70.8[a]	64.0[bc]	60.5[bc]	63.6[bc]	1.19	<0.001
Starch	83.6[c]	90.5[ab]	88.0[b]	90.8[ab]	92.3[ab]	88.3[ab]	90.1[ab]	93.7[a]	1.31	<0.001
GE[3]	62.9[c]	66.2[bc]	63.6[c]	68.7[b]	72.3[a]	66.8[bc]	63.0[c]	66.3[bc]	1.10	<0.001
IDE[4] (kcal/kg)	2,723[d]	2,930[bc]	2,710[d]	3,047[b]	3,196[a]	2,978[b]	2,777[cd]	2,913[bc]	48.12	<0.001
CP	73.3[abc]	76.9[ab]	71.1[bc]	78.1[a]	78.8[a]	75.2[abc]	70.2[c]	73.0[abc]	1.53	0.001
Total tract										
DM	67.1[b]	68.2[b]	67.4[b]	69.4[b]	73.3[a]	69.4[b]	65.4[b]	68.4[b]	1.02	0.001
GE	70.3[bc]	72.6[b]	70.5[bc]	73.4[ab]	76.3[a]	73.2[ab]	68.5[c]	70.9[bc]	0.93	<0.001
AME[5](kcal/kg)	3,043[c]	3,215[b]	3,005[c]	3,254[ab]	3,372[a]	3,260[ab]	3,018[c]	3,118[bc]	40.18	<0.001
CP	53.5[bc]	57.0[ab]	53.6[bc]	56.6[ab]	60.3[a]	58.3[ab]	50.1[c]	57.4[ab]	1.19	<0.001

[1]CPSR: Canada Prairie Spring Red; CWRS: Canada Western Red Spring; CWSWS: Canada Western Soft White Spring; CWHWS: Canada Western Hard White Spring; CWAD: Canada Western Amber Durum; CWRW: Canada Western Red Winter
[2]All means are average of 8 cages per treatment
[3]Gross energy
[4]Ileal digestible energy
[5]Apparent metabolizable energy
[a-d]Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 4.6 Relationships of test sample *in vivo* AME[1] (chick bioassay) with physico-chemical characteristics and growth performance variables

Item	Coefficient of determination (R^2)	*P-value*
Test weight	0.09	0.483
1,000 kernel weight	0.01	0.773
GE[2]	0.80	0.003
Starch	0.35	0.123
Ether extract	0.10	0.458
CP	0.83	0.002
NDF[3]	0.28	0.176
Ileal starch digestibility of diets	0.21	0.259
Growth performance (8-13 d of age)		
BW gain	0.96	<0.001
Feed intake	0.45	0.069
FCR[4]	0.85	0.001

[1]Apparent metabolizable energy
[2]Gross energy
[3]Neutral detergent fiber
[4]Feed conversion ratio

Table 4.7 Regression equations for prediction of *in vivo* AME[1] based on *in vitro* AME and other chemical characteristics of 8 test samples (kcal/kg of DM)

Prediction equations	R^2	SEP[2]	*P*-value
In vivo AME = - 898.14 + 1.1665× *in vitro AME*	0.81	68.6	0.002
In vivo AME = - 1198.1 + 11.606 × NDF[3] + 1.213 × *in vitro* AME	0.81	68.2	0.015
In vivo AME = - 1013.4 - 125.2 × EE[4] + 1.252 × *in vitro* AME	0.83	65.7	0.013
In vivo AME = 929.22 - 21.575 × starch + 1.024 × *in vitro* AME	0.90	50.5	0.003
In vivo AME = 336.35 + 31.836 × CP + 0.6707 × *in vitro* AME	0.98	23.5	<0.001
In vivo AME = -1626.8 - 150.18 × EE + 22.843 × NDF + 1.3606 × *in vitro* AME	0.83	64.4	0.050
In vivo AME = 1235.1 + 67.975 × EE - 24.448 × starch + 0.9586 × *in vitro* AME	0.90	49.8	0.018
In vivo AME = 2154.7-32.946 × NDF - 25.99 × starch + 0.8629 × *in vitro* AME	0.91	47.4	0.015
In vivo AME = 7454.3-120.2 × NDF + 456.38 × EE - 56.971 × starch - 0.0029 × *in vitro* AME	0.96	30.7	0.018
In vivo AME = 2990.3 - 48.755× NDF + 239.83× EE - 14.059 × starch +31.375 ×CP + 0.226× *in vitro* AME	0.99	12.5	0.016

[1]Apparent metabolizable energy
[2]Standard error of prediction (kcal/kg)
[3]Neutral detergent fiber
[4]Ether extract

Table 4.8 Prediction of *in vivo* AME[1] value of test samples based on *in vitro* AME and 4 chemical characteristics (kcal/kg of DM)[2]

Item	*In vivo* AME of test samples (chick bioassay)	Predicted *in vivo* AME of test samples
CPSR	3,109	3,103
CWRS	3,324	3,325
CWSWS	3,061	3,052
CWHWS	3,373	3,394
CWAD	3,520	3,507
CWRW	3,380	3,379
Triticale 2005	3,078	3,097
Triticale 2006	3,203	3,191
Prediction equation	*In vivo* AME = 2990.3 - 48.755× NDF[3] + 239.83× EE[4]-14.059 × starch +31.375 ×CP + 0.226× *in vitro* AME	
R^2	0.99	
SEP[5] (kcal/kg)	12.5	
P value	0.016	

[1]Apparent metabolizable energy
[2]CPSR: Canada Prairie Spring Red; CWRS: Canada Western Red Spring; CWSWS: Canada Western Soft White Spring; CWHWS: Canada Western Hard White Spring; CWAD: Canada Western Amber Durum; CWRW: Canada Western Red Winter
[3]Neutral detergent fiber
[4]Ether extract
[5]Standard error of prediction

Chapter 5

Prediction of response to an NSP-degrading enzyme product on AME of wheat and triticale samples using an *in vitro* digestibility technique

5.1 Introduction

Wheat is an important feedstuff that can contribute to up to 70% of the energy of broiler rations (Veldman and Vahl, 1994). Apparent metabolizable energy (**AME**) values can vary significantly among wheat samples (Rafuse et al., 2005).

Supplementation of wheat-based diets with NSP-degrading enzymes with or without other enzyme activities can reduce these variations and also increase the AME value of the diets. The difference between highest and lowest AME of 108 wheat samples of 9 cultivars was 10% and addition of an NSP-degrading enzyme reduced this difference (Scott et al., 1998a). Responses to enzyme supplementation were different among wheat samples. Enzyme supplementation had a small effect (an average increase of 3.4 %) on AME of Durum wheat samples, however, Canadian Prairie Spring wheats had the greatest response to enzyme addition (an average increase of 8.7 % in AME). This extent of response in Canadian Prairie Spring wheat might be attributed to the presence of higher concentrations of non-starch polysaccharides (**NSP**; Scott et al., 1998a). Rafuse et al. (2005) also reported that a mixture of xylanase and protease enzymes reduced variations in ileal AME of wheat-based diets from 503 kcal/kg (2,823 to 3,326 kcal/kg) to 382 kcal/kg (2,980 to 3,362 kcal/kg). Addition of xylanase and

protease increased the AME of wheat-based diets in broiler chicks and reduced the variations among the diets (Svihus and Gullord, 2002).

Prediction of variations of AME in wheat samples is critical for a more accurate diet formulation. This prediction can be done through measuring physical or chemical characteristics, conducting animal digestibility trials, *in vitro* digestibility techniques, and near infrared reflectance spectroscopy (Yegani and Korver, 2012). *In vitro* digestibility techniques can accurately predict AME of wheat samples in broiler chickens (Chapter 4).

Attempts have been made to predict the effects of exogenous enzymes on nutritive value of cereal samples or diets using *in vitro* digestibility techniques (Bedford and Classen, 1993; Li et al., 2010). This prediction may provide a basis for screening of exogenous enzymes for inclusion in animal diets (Bedford and Classen, 1993; Li et al., 2010). However, there is very limited information available on the possibility of predicting effects of NSP degrading enzymes with or without other enzyme activities on the *in vivo* AME of wheat samples through *in vitro* digestibility methods.

Nutritive values of 7 wheat and 1 triticale samples were evaluated in a chick bioassay (with and without a commercial exogenous enzyme product). The ability of a two-step *in vitro* digestibility technique in predicting enzyme effects on *in vivo* AME of the test samples in broiler chicks was also assessed.

5.2 Materials and methods

5.2.1 Test samples

Seven samples of wheat were obtained from various sources in western Canada. Wheat samples were: Three varieties of Canada Western Red Spring (**CWRS**), two varieties of Canada Western Red Winter (**CWRW**), two varieties of Canada Prairie Spring Red (**CPSR**). One sample of triticale (variety unknown) was also evaluated.

Test weight and 1,000 kernel weight of these samples were measured at the Field Crop Development Center of the Alberta Agriculture and Rural Development (Lacombe, AB, Canada) according to the guidelines of the Canadian Grain Commission (Winnipeg, MB, Canada). The samples were also tested by Avicheck^{TM} Wheat model (Danisco Animal Nutrition, Marlborough, Wiltshire, UK) to determine their *in vitro* viscosity (Cowieson et al., 2005). As briefly described by Cowieson et al. (2005), this is a two-step *in vitro* digestion technique that uses a Brookfield viscometer to measure the viscosity of the test samples at 20 °C, based on the method proposed by Bedford and Classen (1993).

5.2.2 Chick bioassay

This study was approved by the Animal Care and Use Committee: Livestock of the University of Alberta and met guidelines of the Canadian Council on Animal Care (CCAC, 1993). A total of 1,536 one-d-old male broiler chicks (Ross 308 strain, Aviagen, Huntsville, AL) were randomly assigned into groups of 12 chicks to 128 Specht pullet cages (53 × 59 × 44 cm, Specht Canada Inc., Stony Plain, AB, Canada). The room temperature was initially set at 34 ºC and was accordingly decreased by the end of the experiment which was day 14. The lighting program was 23 hr light: 1hr dark per day and the birds had unrestricted access to feed and water throughout the feeding trial.

All birds were fed a wheat-based starter diet from day 0 to 7 of age and then the experimental diets were fed from day 8 to 14. Eight test samples were used to mix a total of 16 experimental diets as two diets for each test sample, with and without exogenous enzyme. As previously described (Scott et al., 1998b), each test sample was included at 80% of the diet and the remaining 20% was a fixed mixture of other ingredients. However, the ingredient composition of the mixture was different from what Scott et al. (1998b) used in their study. The composition of the starter and experimental diets are given in Table 5.1. Celite (Celite Corporation, World Minerals Inc., Lompoc, CA) was included at 1% in all experimental diets as an indigestible ash marker for determination of apparent ileal and total tract digestibility of energy.

A commercial enzyme product (Avizyme 1502; Danisco Animal Nutrition, Marlborough, Wiltshire, UK) was added at the rate of 500 g per tonne into one set of each of the respective diets (i.e., 8 diets out of 16 diets). This product, according to the information disclosed by the manufacturer, provided the following enzyme activities (units per kg of the diet): endo -1, 4-beta-xylanase (EC 3.2.1.8) 2000 units, protease (EC 3.4.21.62) 4000 units, and alpha- amylase (EC 3.2.1.1) 400 units.

All experimental diets were analyzed for xylanase and amylase activities. Protease activity was not tested in the experimental diets of the present study. Enzyme recovery analyses were conducted by Danisco Animal Nutrition (Marlborough, Wiltshire, UK). As briefly described by Cowieson et al. (2006), xylanase activity in the experimental diets was determined by following a

modified method based on the Megazyme xylanase assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Amylase activity in the diets was assessed by using phadebas tablets (Megazyme International Ireland Ltd.), according to the method of Barnes and Blakeney (1974) and McCleary and Sheehan (1989).

5.2.3 Growth performance and digestibility measurements

The experimental diets were fed to the birds in a mash form. Each experimental diet was fed to 8 cages of 12 chicks (96 chicks per diet) from day 8 to 14 of age. Body weight (**BW**) and feed intake were recorded and feed conversion ratio (**FCR**) was calculated for each cage. Cage mortality (number and weight of dead birds within each cage) was recorded daily throughout the experiment and FCR was corrected accordingly at the time of calculating growth performance variables. At 14 d of age, all birds were euthanized by cervical dislocation and contents of the ileum (from Meckel's diverticulum to the ileo-cecal junction) were collected in plastic bags, pooled within each cage, and frozen at -20ºC immediately. Pooled digesta samples were subsequently freeze-dried and ground for laboratory analyses (Garcia et al., 2007). Excreta samples were collected from a tray placed below each cage for a 48 h period from d 12 to d 14. Excreta samples were prepared for chemical analyses as described for the digesta samples.

5.2.4 Chemical analyses

Dry matter (**DM**) contents of test samples, experimental diets, ileal digesta, and excreta samples were determined (method 934.01; AOAC, 2005). Acid

insoluble ash (**AIA**) contents of the samples were determined (McCarthy et al., 1974). Gross energy (GE) of all samples was measured by bomb calorimetry using an adiabatic calorimeter (IKA® Werke GmbH & Co. KG, Staufen, Germany) standardized with benzoic acid (Nortey et al., 2008). Test samples were also analyzed for ether extract (method 920.39; AOAC, 2005), neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**) as described by van Soest et al. (1991). Starch determination in the test samples was done using the total starch assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). This procedure uses a combination of thermostable α-amylase and amyloglucosidase. Nitrogen content of test samples was also determined by combustion with an automatic nitrogen analyzer (Leco TruSpec CN, Leco Corp., St. Joseph, MI), and then multiplied by 6.25 to calculate crude protein (**CP**) content of each test sample (method 968.06; AOAC, 2005).

The apparent ileal and total tract digestibility of DM and energy of the experimental diets were calculated according to the following formulas (Olukosi et al., 2007):

Nutrient digestibility of the diets (%) =

$$\{1-[(AIA_{feed} / AIA_{digesta\ or\ excreta}) \times (Nutrient_{digesta\ or\ excreta} / Nutrient_{feed})]\} \times 100$$

Ileal digestible energy (**IDE**) or AME of the diets (kcal/kg) =

$$GE_{feed} - [GE_{digesta\ or\ excreta} \times (AIA_{feed} / AIA_{digesta\ or\ excreta})]$$

Determined dietary AME values were then used to calculate AME of test samples as described by Scott et al. (1998b). Based on the values reported in the

NRC (1994), the total energy contribution of the fixed ingredients that made 20% of the diet was 555.9 kcal/kg.

AME of the test sample (kcal/kg) =

$$(\text{AME of the experimental diet} - 555.9) \times 100/80$$

Dietary IDE is a measure of the amount of energy absorbed by the bird up to the ileum, minimizing the confounding effects of hindgut microbiota on energy measurement using excreta samples (Olukosi et al., 2007).

5.2.5 In vitro digestibility technique

A two-step *in vitro* digestibility technique (Chapter 4) was used to predict *in vivo* AME of 8 test samples (both with and without exogenous enzyme) for broiler chickens. This *in vitro* technique simulated the gastric and post-gastric phases. The enzyme product (Avizyme 1502) was also added (at the same rate used in the chick bioassay) to each flask in the gastric phase according to the technical instructions provided by the laboratory staff of the enzyme manufacturer (M. Faurschou Isaksen, Danisco, Brabrand, Aarhus, Denmark, personal communication). The *in vitro* AME of the test samples was calculated as described previously (Chapter 4).

5.2.6 Statistical analyses

Growth performance and apparent ileal and total tract digestibility of energy were analyzed using the Mixed procedure of SAS to examine the main effects of cereal grain, exogenous enzyme, and their interaction (SAS Institute, 2002). Cage was considered as the experimental unit. Means were separated by using the

probability of difference (**Pdiff**) option of least square means. Differences were considered significant at $P \leq 0.05$.

The regression procedure of SAS (SAS Institute, 2002) was used to investigate the relationships between different parameters. Coefficient of determination (R^2) and standard error of prediction (**SEP**) were used to determine the quality of prediction of *in vivo* AME. Equations from Chapter 4 were used to predict *in vivo* AME of the cereal samples and also to determine SEP.

Variations in different characteristics of wheat samples can be classified into 3 groups (Jha et al., 2011; Chapter 4): 1) wide variation, CV > 10%; 2) medium variation, CV from 5 to 10%, and 3) small variation, CV < 5%. This classification will help to explain variations in physico-chemical characteristics of 8 test samples.

5.3 Results

5.3.1 Physico-chemical characteristics of test samples

The 1,000 kernel weight varied (CV= 9.8%) among test samples and ranged from 34 g for CRWS (Harvest) and CWRW (Falcon) to 44 g for CWRS (Superb) wheat. The CV for test weight (kg/hL) was small (CV=3.2%) and varied from 75 kg/hL for CWRS (Harvest) to 81.6 kg/hL for CWRW (AC Bellatrix) wheat (Table 5.2).

The CV for DM (CV= 0.8%), GE (CV=0.7%), and starch (CV= 4.1%) were small (Table 5.2). However, CP (CV= 10.9%), ether extract (CV= 14.8%), NDF (CV= 10.7%), ADF (CV=11.3%), and *in vitro* viscosity (CV= 19.8%) contents varied widely. The CP content varied from 15.8% in CWRW (Falcon) to

22.0% in CWRS (Lillian). Ether extract content ranged from 1.4% in Triticale and CWRW (AC Bellatrix) to 2.1% in CWRW (Falcon). The CWRS (Superb) had the lowest (11.2%) and Triticale had the highest (14.5%) NDF contents, respectively. The *in vitro* viscosity also varied widely among test samples and ranged from 3.96 cp in Triticale to 6.74 cp in CWRW (Falcon).

The analyzed chemical contents of experimental diets are presented in Table 5.3. Table 5.4 shows the activities of exogenous enzymes recovered from 16 each of the experimental diets. The recovered amylase was substantially high (at least 15 times higher) in non-enzyme supplemented triticale diet.

5.3.2 Growth performance variables

Cereal grain had a significant effect on BW gain ($P < 0.001$), feed intake ($P = 0.041$), and FCR ($P < 0.001$) of birds fed experimental diets, however, the effects of enzyme (except for FCR; $P = 0.044$) and enzyme × cereal grain interactions were not significant (Table 5.5). Birds fed CWRW (Falcon) and CWRS (Lillian)-based diets had the lowest and highest BW gain, respectively. Birds fed Triticale diet had the lowest and CPRS (AC Crystal) had the highest feed intake. The lowest FCR was observed in birds fed CWRS (Lillian) diet, whereas, the highest FCR was in CWRW (Falcon)-fed group of birds. As mentioned above, enzyme had a significant effect ($P = 0.044$) on FCR, improving it by 2.0%.

5.3.3 In vivo digestibility

Both cereal grain ($P < 0.001$) and enzyme ($P < 0.001$) had significant effects on ileal digestibility of dietary DM, GE, and IDE. However, there were no

cereal grain × enzyme interactions (Table 5.6). The CWRS (Superb) and CWRW (Falcon) had the lowest and highest ileal DM and GE digestibility, respectively. However, CWRS (Lillian) had the highest IDE. Enzyme increased DM and GE ileal digestibility and also IDE by 6.0, 5.7, and 5.5%, respectively.

Effects of cereal grain ($P < 0.001$) and enzyme ($P < 0.001$) were significant on the total tract digestibility of dietary DM, GE, and AME (Table 5.6). The cereal grain × enzyme interactions were not significant. The total tract digestibility of DM and GE, AME were the lowest in CWRS (Superb) and the highest in CPSR (AC Crystal). Enzyme enhanced digestibility of DM, GE and AME of diets by 4.6, 4.3, and 4.2%, respectively.

The AME of the test samples were calculated from dietary AME. The calculated AME of the samples (from diets without enzyme) ranged from 3,110 for CPSR (CPS-5700) and CWRS (Superb) to 3,386 kcal/kg for CPSR (AC Crystal) sample (Table 5.7). The calculated AME of the test samples (in enzyme supplemented diets) were from 3,265 kcal/kg for CWRS (Superb) to 3,535 kcal/kg for CWRS (Lillian).

5.3.4 Relationship of test sample AME with other characteristics

There was no relationship between the test sample *in vivo* AME (without enzyme) and test weight or 1,000 kernel weight ($R^2 = 0.01$ and 0.11, respectively; Table 5.8). The AME of the test samples was also not related with GE ($R^2 = 0.17$; $P = 0.308$), starch ($R^2 = 0.25$; $P = 0.207$), ether extract ($R^2 = 0.01$; $P = 0.805$), CP ($R^2 = 0.22$; $P = 0.242$), NDF ($R^2 = 0.08$; $P = 0.493$), ADF ($R^2 = 0.02$; $P = 0.761$) or *in vitro* viscosity ($R^2 = 0.04$; $P = 0.639$). There was also no relationship between

AME and BW gain, feed intake, or FCR of the birds during 8 to 14 day of age. The AME of enzyme-supplemented samples also followed the same pattern, however, the only difference was a significant relationship between the AME and feed intake ($R^2 = 0.54$; $P = 0.037$).

5.3.5 In vitro digestibility

Prediction of *in vivo* AME (without exogenous enzyme) of the test samples using the same equations as Chapter 4 are given in Table 5.9. Predicted AME values for enzyme supplemented samples (using equations from Chapter 4) are presented in Table 5.10.

5.4 Discussion

The AME content of the test samples, with or without enzyme, were not related with their physical characteristics in accordance with previous report (Chapter 4). This lack of relationship was also observed with chemical characteristics of the test samples. Our previous observations (Chapter 4) showed a relationship between AME of wheat and triticale samples with GE and CP, however, similar to the present study, there was no relationship between AME and starch, ether extract and NDF. The reasons for discrepancies in our observations in terms of relationship between AME and chemical characteristics in these two studies are not clearly known, however, this indicates that physical characteristics do not accurately predict the nutritive value of feedstuffs as discussed in Chapter 4

The *in vitro* viscosity of the test samples in the present study were within a relatively similar range as in previous studies (Pirgozliev et al., 2001; Carre et al., 2002). Interestingly, the *in vitro* viscosity was not related to the AME of the cereal grains and this is in contrast with other studies which reported a negative relationship between the *in vitro* viscosity with dietary AME (Carre et al., 2002) or the ratio of energy retention to AMEn intake (Pirgozliev et al., 2001). However, our observation is in line with Svihus and Gullord (2002) who also did not find any relationship between wheat viscosity and the feeding value of wheat-based diets with a 77% inclusion rate of wheat.

The cereal grain had a significant effect on performance variables, although enzyme (except for FCR) and cereal grain × enzyme interactions did not affect these variables in the present study. This is in accordance with other studies as well (Rafuse et al., 2005; Gutierrez del Alamo et al., 2008). Gutierrez del Alamo et al. (2008) reported that addition of a mixture of xylanase and protease had no effect on performance variables, but the effect of wheat cultivar was significant. Rafuse et al. (2005) observed that a mixture of xylanase and protease had no effect on performance possibly due to the low viscosity of wheat samples (4.58 to 17.59 centipoise), resulting in lower responses to enzyme supplementation. The lack of enzyme response on performance variables in the present study might therefore also be explained by low *in vitro* viscosity of the test samples which even had a narrower range (3.96 to 6.74 centipoise) compared to samples in the study of Rafuse et al. (2005).

The AME of the test samples without enzyme ranged from 3,110 to 3,386 kcal/kg (8.9% difference). Enzyme supplementation had little effect on narrowing this range as AME varied from 3,265 to 3,535 kcal/kg (8.3% difference). In spite of this, enzyme supplementation increased AME value of across all test samples in the present study. Other studies have also reported positive effects of exogenous enzyme on AME value of wheat samples (Bedford et al., 1998; Scott et al., 1998a; Rafuse et al., 2005). AME content of 108 wheat samples ranged from 3,280 to 3,650 kcal kg (a variation of 11%). Enzyme supplementation reduced this variation to 5% (3,600 to 3,780 kcal kg, Scott et al., 1998a). Addition of a mixture of xylanase and protease enzymes reduced variations in ileal AME of wheat-based diets from 503 kcal/kg (2,823 to 3,326 kcal/kg) to 382 kcal/kg (2,980 to 3,362 kcal/kg, Rafuse et al. (2005).

The increase in the AME value ranged from 1.5% for CWRS (Harvest) to 8.8% for CPSR (CPS-5700). Response of wheat to enzyme supplementation is dependent on the nutritive value of the sample (Scott et al., 1998a). Wheat samples that have lower nutritive value respond to a greater extent to enzyme supplementation than wheat samples that have higher nutritive value. The NSP content of wheat and triticale samples were not determined in the present study, but NSP concentrations can play an important role with respect to responses to exogenous enzymes. Lower responses to enzymes might be an indication of lower NSP levels in a given wheat sample (Scott et al., 1998a). This may explain as to why the extent of response to enzyme supplementation in CWRS (Harvest) was lower than CPSR (CPS-5700).

Regression equations developed in Chapter 4 were used to predict AME values of the test samples. The quality of prediction was not good compared to the study in Chapter 4. Variability in in vivo AME values of the test samples in the present study was low which may have contributed to this situation. Boisen and Fernandez (1995) reported that a narrow range in in vivo values can, to some extent, be responsible for a low relationship between the actual and predicted digestibility values in pigs. Regmi et al. (2008, 2009) also suggested that it is critical that samples used in in vitro assays represent a wide range in nutritional quality.

Another aspect of the present study was to investigate if the *in vitro* digestibility technique could also predict enzyme effect on the AME of the test samples. The in vitro technique did not predict enzyme response on AME. The lack of ability of *in vitro* digestibility techniques in predicting enzyme response has been reported by Li et al. (2010) as well. These researchers used a two-step *in vitro* digestibility technique to investigate the effects of different levels of a mixture of xylanase, amylase, and protease on apparent digestibility of nutrients of corn-soy diets under both *in vitro* and *in vivo* conditions in pigs. However, it was concluded that the ability of the *in vitro* digestibility assay was limited in predicting the response of animals such as poultry and pigs to exogenous enzyme products.

The possible factors responsible for the lack of effects are not clearly known, but it is suggested that the lack of effect of the exogenous enzyme under

in vitro environment might indicate that there were not proper conditions allowing the exogenous enzyme product to work.

In conclusion, the results showed that the exogenous enzyme product increased AME of all the test samples, however, the *in vitro* digestibility assay did not predict the enzyme response on AME value of the test samples. In addition to low variability in in vivo AME values as a contributing factor, this situation might indicate that the in vitro conditions required for prediction of exogenous enzyme response may be different from conditions where no exogenous enzyme product is used in the in vitro environment.

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Table 5.1 Composition of the experimental diets fed to broiler chicks

Ingredients (%)	Starter (day 0 to 7)	Experimental diet (day 8 to 14)
Corn	18.00	0.00
Vegetable fat	3.76	2.00
Fish Meal	3.00	3.00
Soybean meal	26.79	8.00
Wheat	43.41	80.00
Calcium carbonate	1.32	1.22
Dicalcium phosphate	1.50	1.55
Salt	0.42	0.35
L-Lysine	0.23	0.83
DL-Methionine	0.23	0.36
L-Threonine	0.05	0.35
Vitamin E 5,000 IU/Kg	0.30	0.30
Enzyme[1]	0.00	±
Celite	0.00	1.00
Vit-min premix[2]	0.5	0.5
Choline chloride[3]	0.5	0.5
Calculated nutrient content		
Protein	23.00	18.00
Metabolizable energy (kcal/kg)	3,079	3,044
Calcium	1.1	1.05
Available P	0.5	0.5

[1]Avizyme 1502, Danisco Animal Nutrition, Marlborough, Wiltshire, UK (0.5 g of enzyme per kg of diet provided 2000, 4000, 400 units of xylanase, amylase, and protease, respectively)
[2]Vitamin/mineral premix provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 ICU; vitamin E, 35 IU; menadione, 2.0 mg; D-pantothenic acid, 14 mg; riboflavin, 5.0 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18; iodine, 0.5 mg; manganese, 70 mg; copper, 8.5 mg; zinc, 80 mg; selenium, 0.1 mg; iron, 100 mg
[3]Provided 100 mg of choline per kilogram of diet

Table 5.2 Analyzed physical characteristics and chemical composition of test samples[1] (%) - DM basis

Item	Triticale	CWRS (Harvest)	CWRS (Superb)	CWRS (Lillian)	CWRW (Falcon)	CWRW (AC Bellatrix)	CPSR (AC Crystal)	CPSR (CPS-5700)	CV[2] (%)
Test weight (kg/hL)	NA[3]	75.0	80.4	78.5	76.2	81.6	77.9	75.3	3.2
1,000 kernel weight (g)	NA	34.0	44.0	38.0	34.0	37.0	41.5	40.0	9.8
DM	87.9	88.7	87.9	88.2	87.4	88.0	88.3	86.3	0.8
CP	18.2	19.5	16.7	22.0	15.8	16.4	18.4	17.7	10.9
GE[4] (kcal/kg)	4,443	4,455	4,415	4,492	4,419	4,389	4,427	4,425	0.7
Starch	58.6	60.5	65.2	59.1	63.3	65.1	61.9	61.3	4.1
Ether extract	1.4	1.8	1.6	1.8	2.1	1.4	1.5	1.6	14.8
NDF[5]	14.5	14.0	11.2	12.0	14.4	11.6	11.7	12.3	10.7
ADF[6]	4.0	3.5	3.1	3.1	3.6	3.0	2.9	3.2	11.3
In vitro viscosity (cp)[7]	3.96	4.23	5.24	4.57	6.74	6.07	4.66	4.25	19.8

CWRS: Canada Western Red Spring, CPSR: Canada Prairie Spring Red, CWRW: Canada Western Red Winter
[2]Coefficient of variation
[3]Not available
[4]Gross energy
[5]Neutral detergent fiber
[6]Acid detergent fiber
[7]Centipoise

Table 5.3 Chemical analyses of experimental diets (%) - DM basis

Diets	DM (%)		GE[1] (kcal/kg)	
	without enzyme	with enzyme	without enzyme	with enzyme
Triticale	89.0	89.1	4,331	4,331
CWRS[2] (Harvest)	89.7	89.6	4,340	4,339
CWRS (Superb)	89.3	89.3	4,305	4,318
CWRS (Lillian)	89.3	89.4	4,379	4,376
CWRW[3] (Falcon)	89.0	88.8	4,311	4,314
CWRW (AC Bellatrix)	89.3	89.0	4,314	4,288
CPSR[4] (AC Crystal)	89.4	89.4	4,339	4,328
CPSR (CPS-5700)	88.1	88.0	4,307	4,306

[1]Gross energy
[2]Canada Western Red Spring
[3]Canada Western Red Winter
[4]Canada Prairie Spring Red

Table 5.4 Enzyme activities in experimental diets (units/kg feed)[1]

Diets	Xylanase		Amylase	
	without enzyme	with enzyme	without enzyme	with enzyme
Triticale	<100	3,868	2,477	4,607
CWRS[2] (Harvest)	<100	1,987	160	1,371
CWRS (Superb)	<100	4,426	NA[3]	1,047
CWRS (Lillian)	<100	2,768	209	2,102
CWRW[4] (Falcon)	<100	2,305	235	1,877
CWRW (AC Bellatrix)	<100	4,456	191	1,111
CPSR[5] (AC Crystal)	<100	3,551	188	1,843
CPSR (CPS-5700)	NA	2,636	217	1,287

[1]Determined by Danisco Animal Nutrition, Marlborough, Wiltshire, UK (Protease activity was not determined in the experimental diets)
[2]Canada Western Red Spring
[3]Not available
[4]Canada Western Red Winter
[5]Canada Prairie Spring Red

Table 5.5 Growth performance of broiler chicks fed experimental diets (day 8 to 14)[1]

Item	BW gain (g/bird/d)	Feed intake (g/bird/d)	FCR[2](g feed : g gain)
Cereal grain effect			
Triticale	24.2[cde]	48.0[b]	2.01[cd]
CWRS[3] (Harvest)	27.0[abc]	50.6[ab]	1.88[e]
CWRS (Superb)	25.1[bcd]	52.4[ab]	2.09[bc]
CWRS (Lillian)	28.8[a]	50.1[ab]	1.74[f]
CWRW[4] (Falcon)	22.0[e]	49.8[ab]	2.28[a]
CWRW (AC Bellatrix)	23.5[de]	50.2[ab]	2.14[b]
CPSR[5] (AC Crystal)	27.7[ab]	52.5[a]	1.91[de]
CPSR (CPS-5700)	24.1c[de]	50.0[ab]	2.10[bc]
Pooled SEM	0.68	0.98	0.03
Enzyme effect			
-	25.1	50.6	2.04[a]
+	25.5	50.3	2.00[b]
Pooled SEM	0.34	0.49	0.01
P-values			
Cereal grain	<0.001	0.041	<0.001
Enzyme	0.520	0.667	0.044
Cereal grain × Enzyme	0.666	0.488	0.829

[1]All means are average of 8 cages per treatment
[2]Feed conversion ratio
[3]Canada Western Red Spring
[4]Canada Western Red Winter
[5]Canada Prairie Spring Red
Means within a column with no common superscripts are significantly different ($P \leq 0.05$)

Table 5.6 Apparent ileal and total tract digestibility of energy of experimental diets (%) [1] - DM basis

Item	Ileal			Total tract		
	DM (%)	GE[2] (%)	IDE[3] (kcal/kg)	DM (%)	GE (%)	AME[4] (kcal/kg)
Cereal grain effect						
Triticale	68.6[ab]	71.2[ab]	3,082[ab]	70.8[a]	75.3[ab]	3,261[a]
CWRS[5] (Harvest)	66.1[abc]	69.0[abc]	2,992[abc]	69.0[ab]	74.1[abc]	3,214[ab]
CWRS (Superb)	64.3[c]	67.4[c]	2,904[c]	67.0[b]	72.0[c]	3,106[c]
CWRS (Lillian)	68.0[ab]	71.2[ab]	3,117[a]	69.6[ab]	75.2[ab]	3,292[a]
CWRW[6] (Falcon)	69.3[a]	71.8[a]	3,097[a]	70.6[a]	75.2[ab]	3,244[ab]
CWRW (AC Bellatrix)	67.8[abc]	70.7[ab]	3,040[abc]	69.2[ab]	74.5[abc]	3,202[abc]
CPSR[7] (AC Crystal)	68.7[ab]	71.5[ab]	3,098[a]	71.1[a]	76.0[a]	3,295[a]
CPSR(CPS-5700)	65.5[bc]	68.4[bc]	2,947[bc]	68.0[b]	73.2[bc]	3,153[bc]
Pooled SEM	0.78	0.73	31.55	0.57	0.54	23.25
Enzyme effect						
-	65.3[b]	68.2[b]	2,953[b]	67.9[b]	72.9[b]	3,154[b]
+	69.2[a]	72.1[a]	3,116[a]	71.0[a]	76.0[a]	3,288[a]
Pooled SEM	0.39	0.37	15.78	0.29	0.27	11.62
P-values						
Cereal grain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Enzyme	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cereal grain × Enzyme	0.202	0.211	0.171	0.060	0.102	0.089

[1]All means are average of 8 cages per treatment
[2]Gross energy
[3]Ileal digestible energy
[4]Apparent metabolizable energy
[5]Canada Western Red Spring
[6]Canada Western Red Winter
[7]Canada Prairie Spring Red
[a-c]Means within a column with no common superscripts are significantly different ($P \leq 0.05$)

Table 5.7 Effects of enzyme supplementation on AME[1,2] of test samples determined in the bioassay (kcal/kg) - DM basis

Cereal grain	AME (kcal/kg)		% difference compared to without enzyme
	without enzyme	with enzyme	
Triticale	3,304	3,459	+ 4.7
CWRS[3] (Harvest)	3,299	3,348	+ 1.5
CWRS (Superb)	3,110	3,265	+ 5.0
CWRS (Lillian)	3,305	3,535	+ 7.0
CWRW[4] (Falcon)	3,239	3,481	+ 7.5
CWRW (AC Bellatrix)	3,231	3,385	+ 4.8
CPSR[5] (AC Crystal)	3,386	3,461	+ 2.2
CPSR (CPS-5700)	3,110	3,384	+ 8.8

[1]Apparent metabolizable energy
[2]Calculated from dietary AME as described by Scott et al. (1998b)
[3]CWRS: Canada Western Red Spring
[4]CWRW: Canada Western Red Winter
[5]CPSR: Canada Prairie Spring Red

Table 5.8 Relationships of *in vivo* AME[1] (with and without enzyme) with physico-chemical characteristics and growth performance variables

Item	R^2		*P*-value	
	without enzyme	with enzyme	without enzyme	with enzyme
Test weight	0.01	0.03	0.836	0.692
1,000 kernel weight	0.11	0.14	0.475	0.417
GE[2]	0.17	0.24	0.308	0.215
Starch	0.25	0.33	0.207	0.137
Ether extract	0.01	0.03	0.805	0.695
CP	0.22	0.19	0.242	0.286
NDF[3]	0.08	0.09	0.493	0.464
ADF[4]	0.02	0.02	0.761	0.736
In vitro viscosity	0.04	0.00	0.639	0.973
BW gain (d 8 to 14)	0.41	0.13	0.087	0.372
Feed intake (d 8 to 14)	0.10	0.54	0.452	0.037
FCR[5](d 8 to 14)	0.36	0.06	0.119	0.555

[1]Apparent metabolizable energy
[2]Gross energy
[3]Neutral detergent fiber
[4]Acid detergent fiber
[5]Feed conversion ratio

Table 5.9 Regression equations for prediction of *in vivo* AME[1] (without enzyme) based on *in vitro* AME and other chemical characteristics of 8 test samples (kcal/kg of DM)

Prediction equations	R^2	SEP[2]	*P*-value	Mean AME predicted	Mean AME measured
In vivo AME = - 898.14 + 1.1665× *in vitro AME*0.05	0.05	95.7	0.612	3,238	3,248
In vivo AME = - 1198.1 + 11.606 × NDF[3] + 1.213 × *in vitro* AME	0.11	88.4	0.432	3,250	3,248
In vivo AME = - 1013.4 - 125.2 × EE[4] + 1.252 × *in vitro* AME	0.06	100.1	0.548	3,219	3,248
In vivo AME = 929.22 - 21.575 × starch + 1.024 × *in vitro* AME	0.22	89.9	0.243	3,225	3,248
In vivo AME = 336.35 + 31.836 × CP + 0.6707 × *in vitro* AME	0.17	103.0	0.306	3,290	3,248
In vivo AME = -1626.8 - 150.18 × EE + 22.843 × NDF + 1.3606 × *in vitro* AME	0.24	80.3	0.221	3,239	3,248
In vivo AME = 1235.1 + 67.975 × EE - 24.448 × starch + 0.9586 × *in vitro* AME	0.20	91.5	0.261	3,234	3,248
In vivo AME = 2154.7-32.946 × NDF - 25.99 × starch + 0.8629 × *in vitro* AME	0.10	119.4	0.446	3,188	3,248
In vivo AME = 7454.3-120.2 × NDF + 456.38 × EE - 56.971 × starch - 0.0029 × *in vitro* AME	0.01	185.7	0.831	3,148	3,248
In vivo AME = 2990.3 - 48.755× NDF + 239.83× EE - 14.059 × starch +31.375 ×CP + 0.226× *in vitro* AME	0.04	126.0	0.616	3,267	3,248

[1]Apparent metabolizable energy
[2]Standard error of prediction (kcal/kg)
[3]Neutral detergent fiber
[4]Ether extract

Table 5.10 Regression equations for prediction of *in vivo* AME[1] (with enzyme) based on *in vitro* AME and other chemical characteristics of 8 test samples (kcal/kg of DM)

Prediction equations	R^2	SEP[2]	*P*-value	Mean AME predicted	Mean AME measured
In vivo AME = - 898.14 + 1.1665× *in vitro AME*	0.02	96.4	0.761	3,236	3,415
In vivo AME = - 1198.1 + 11.606 × NDF[3] + 1.213 × *in vitro* AME	0.00	87.7	0.930	3,249	3,415
In vivo AME = - 1013.4 - 125.2 × EE[4] + 1.252 × *in vitro* AME	0.04	104.1	0.653	3,217	3,415
In vivo AME = 929.22 - 21.575 × starch + 1.024 × *in vitro* AME	0.18	82.1	0.302	3,224	3,415
In vivo AME = 336.35 + 31.836 × CP[5] + 0.6707 × *in vitro* AME	0.09	98.7	0.464	3,289	3,415
In vivo AME = -1626.8 - 150.18 × EE + 22.843 × NDF + 1.3606 × *in vitro* AME	0.01	83.1	0.852	3,237	3,415
In vivo AME = 1235.1 + 67.975 × EE - 24.448 × starch + 0.9586 × *in vitro* AME	0.25	81.5	0.209	3,233	3,415
In vivo AME = 2154.7-32.946 × NDF - 25.99 × starch + 0.8629 × *in vitro* AME	0.06	115.8	0.558	3,187	3,415
In vivo AME = 7454.3-120.2 × NDF + 456.38 × EE - 56.971 × starch - 0.0029 × *in vitro* AME	0.12	185.8	0.393	3,148	3,415
In vivo AME = 2990.3 - 48.755× NDF + 239.83× EE - 14.059 × starch +31.375 ×CP + 0.226× *in vitro* AME	0.10	184.0	0.436	3,266	3,415

[1]Apparent metabolizable energy
[2]Standard error of prediction (kcal/kg)
[3]Neutral detergent fiber
[4]Ether extract

Chapter 6

Evaluating variations in nutrient availability of field peas and barley samples for broiler chickens: A chick bioassay

6.1 Introduction

With the current volatility in the animal feed market and increasing trend in the price of feed ingredients, it is becoming more important to diversify the ingredient composition of animal diets at commercial levels. This strategy will not only be of help in terms of reducing feed costs, but it can also provide more options for the feed industry when it comes to dealing with challenges associated with the shortage in supplying feed ingredients (Patience et al., 2009).

Corn-soy or wheat-soy based diets are commonly used in poultry feeding in different parts of the world. However, diversion of substantial amounts of grains to the biofuel industry and also competition with human food sector has complicated the animal feed market (Patience et al., 2009). On the other hand, soybean meal is also an expensive source of protein and in some regions such as western Canada, it has to be imported in order to be included in poultry diets (Igbasan and Guenter, 1996a; Nalle et al., 2011). Using alternative ingredients which are less expensive than corn, wheat, or soybean meal can be of help in reducing feed costs in the poultry industry (Savage et al., 1986). Field peas (Ravindran et al., 2009; Nalle et al., 2011) and barley (Brake et al., 1997) are amongst the ingredients that can be considered as alternative feedstuffs for poultry rations.

Incorporation of peas in animal rations has increased in Canada (mainly in western Canada), Europe, and Australia. Peas are also being used in the animal industry in some Asian and Latin American countries (Hickling, 2003). Although peas are a good source of protein and energy (Igbasan and Guenter, 1996b; Nalle et al., 2011), their potential benefits in poultry nutrition has not been fully explored (Igbasan and Guenter, 1996b).

When the prices of other feed ingredients are high, barley can be used in feeding of broiler chickens (Brake et al., 1997). Barley is resistant to drought and as a result, it can be cultivated in many parts of the world (Svihus and Gullord, 2002). However, high soluble fiber content (mainly β-glucans) of barley is a limiting factor for the inclusion of this ingredient in poultry rations (Svihus and Gullord, 2002). This problem can be ameliorated by the use of fiber-degrading exogenous enzymes particularly at younger ages of birds (Jeroch and Danicke, 1995; Svihus and Gullord, 2002). In addition, barley (without the use of exogenous enzymes) can be included up to 20% in the grower and finisher phases in broiler chickens without any negative effects on growth performance of the birds. It was also suggested that inclusion of barley into broiler diets can even be started before 21 day of age (Brake et al., 1997).

One of the major challenges regarding using peas and barley is that they are highly variable in their nutritional values (Fairbairn et al., 1999; Wiseman et al., 2003). Variations in nutrient contents can result in less accurate diet formulation, influencing the performance of the animals (Villamide et al., 1997; Fairbairn et

al., 1999). Thus, it is important to predict variations in nutrient availability of feed ingredients in an efficient manner (Yegani and Korver, 2012; Chapter 4).

This chapter presents two studies that were conducted to further evaluate the nutritional quality of field peas and barley so that this information can be used by the feed industry in western Canada in particular, and the rest of Canada in general. The information presented in this chapter relates to physico-chemical characteristics of eight samples of field peas or barley and also *in vivo* digestibility trials (chick bioassays) determining the feeding values of these samples for broiler chicks.

6.2 Materials and methods

6.2.1 Field pea and barley samples

Field pea and barley samples (eight samples of each ingredient) were obtained from various farms in western Canada. Varieties of field pea samples were of either green or yellow color: Sample 1 (Admiral, yellow), sample 2 (Bluebird, green), sample 3 (Toledo, green), sample 4 (Guard, green), sample 5 (Unknown variety, yellow), sample 6 (Delta, yellow), sample 7 (Stratus, green), and sample 8 (Wadena, yellow). Barley samples were either hulled or hulless: Sample 1 (Harper, hulled, 6 row), sample 2 (CDC McGuire, hulless, 2 row), sample 3 (Helgason, hulled, 6 row), sample 4 (Metcalfe, hulled, 2 row), sample 5 (Tercel, hulless, 2 row), sample 6 (hulled, from breeding plots of Field Crop Development Center, Lacombe, AB), sample 7 (hulless, from breeding plots of Field Crop Development Center, Lacombe, AB), and sample 8 (Ponoka, hulled, 6 row).

Test weight and 1,000 kernel weight of barley samples were measured at the Field Crop Development Center of Alberta Agriculture and Rural Development (Lacombe, AB, Canada) according to the guidelines of the Canadian Grain Commission (Winnipeg, MB, Canada).

6.2.2 Chick bioassays

The studies were approved by the Animal Care and Use Committee: Livestock of the University of Alberta and met guidelines of the Canadian Council on Animal Care (CCAC, 1993). A total of 768 one-d-old male broiler chicks (Ross 308, Aviagen, Huntsville, AL) were randomly assigned into groups of 12 chicks to each of 64 Specht pullet cages (53 × 59 × 44 cm, Specht Canada Inc., Stony Plain, AB, Canada) for field pea samples. The same experimental design was used for the subsequent study with barley samples. The room temperature was initially set at 34 ºC, and was accordingly decreased by the end of the experiments at 13 d of age. The lighting program was 23 hr light: 1hr dark per day and the birds had unrestricted access to feed and water throughout the feeding trials.

All birds in these two studies were fed a wheat-based starter diet from 0 to 7 d of age, and experimental diets were then fed from 8 to 13 d of age. Field pea samples (eight samples) varying in nutrient contents were used to mix 8 experimental diets. The same approach was taken for the 8 barley samples. As previously described by Scott et al. (1998), each sample of field pea or barley was included at 80% of the diet and the remaining 20% was a fixed mixture of other ingredients including soybean meal and fish meal. The ingredient composition of

the mixture used in the present study was different from what Scott et al (1998a) used in their study with barley samples. The ingredient composition and calculated nutrient profile of the diets are given in Table 6.1. Celite (Celite Corporation, World Minerals Inc., Lompoc, CA) was included into all experimental diets as an indigestible ash marker for determination of apparent ileal and total tract digestibility of nutrients and energy. In the barley experiment, chromic oxide was also added as a secondary marker into the diets (in addition to Celite), however, diet, digesta, and fecal samples were not analyzed for chromic oxide. The experimental diets were fed to the birds in a mash form.

6.2.3 Growth performance and digestibility measurements

In both studies, each experimental diet was fed to 8 cages of 12 chicks (96 chicks per diet) from day 7 to 13 of age. Body weight (**BW**) and feed intake were recorded and feed conversion ratio (**FCR**) was calculated for each cage. Mortality (number and weight of dead birds within each cage) was recorded daily throughout the experiment and FCR was corrected accordingly at the time of calculating growth performance variables. At day 13 of age, all birds within each cage were euthanized by cervical dislocation and contents of the ileum (from Meckel's diverticulum to the ileo-cecal junction) was collected in plastic bags, pooled within each cage, and frozen immediately. Pooling of digesta within each pen was done to ensure that sufficient amount of digesta will be available for chemical analyses. Pooled digesta samples were subsequently freeze-dried and ground for laboratory analyses (Garcia et al., 2007). Excreta samples were collected from a tray placed below each cage for a 48 h period from d 11 to d 13.

Excreta samples were prepared for chemical analyses as described for the digesta samples.

6.2.4 Chemical analyses

Dry matter (**DM**) content of test samples, experimental diets, ileal digesta, and excreta samples were determined (method 934.01; AOAC, 2005). Acid insoluble ash (**AIA**) contents of the samples were determined (McCarthy et al., 1974). Gross energy (**GE**) of all samples was measured by bomb calorimetry using an adiabatic calorimeter (IKA® Werke GmbH & Co. KG, Staufen, Germany) standardized with benzoic acid (Nortey et al., 2008). Test ingredient samples were analyzed for ash (method 942.05; AOAC, 2005), ether extract (method 920.39; AOAC, 2005), and neutral detergent fiber (**NDF**, Van Soest et al., 1991). Starch determination in test ingredients, experimental diets, and digesta samples was done using the total starch assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). This procedure uses a combination of thermostable α-amylase and amyloglucosidase. The β-glucan concentrations of barley samples was determined using Megazyme enzyme assay kit as described by McCleary and Glennie-Holmes (1985). Pentosan content was determined according to a modified method of Hashimoto et al. (1987).

Nitrogen content of samples was determined by combustion with an automatic nitrogen analyzer (Leco TruSpec CN, Leco Corp., St. Joseph, MI), and then multiplied by 6.25 to calculate crude protein (**CP**) content of each sample (method 968.06; AOAC, 2005). Amino acid (**AA**) content of test ingredients, experimental diets, and digesta samples were determined through standard

hydrolysis by hydrolyzing approximately 100 mg of each sample with 6M HCl for 24 h at 110 °C. This hydrolysis will allow the release of AA from protein molecules. Amino acids were subsequently separated and quantified on a Varian HPLC instrument (Varian Prostar 210 pump and 410 autosampler, Varian Inc., Palo Alto, CA) and a Varian Fluorichrom fluorescence detector. A reverse phase column (Supelcosil 3 micron LC-18, 4.6 × 150 mm) was used for separation of AA. The samples were derivatized with o-Phthaldialdehyde before injection. Beta-amino-n-butyric acid and ethanolamine were used as internal standards. The acquisition and integration of chromatograms was done by Galaxie software (Galaxie Chromatography Data System, Varian Inc., Palo Alto, CA). In the standard hydrolysis procedure, all amino acids were quantified except Cys, Met, Trp, and Pro (Sedgwick et al., 1991; method 994.12; AOAC, 2005; Cowieson and Ravindran, 2008).

The Cys and Met were determined as cysteic acid and methionine sulfone using the performic acid oxidation method. Cold performic acid (a 1:9 mixture of 88% formic acid: 30% peroxide oxygen) was added to samples and they were oxidized in the fridge overnight. Subsequently, performic acid was eliminated by adding sodium meta-bisulfite and intermittently vortexed for at least 2 hr prior to starting hydrolysis with 6M HCL for a 24 hr period (Sedgwick et al., 1991; method 994.12; AOAC, 2005; Cowieson and Ravindran, 2008b). Tryptophan and proline were not determined in the present study.

The apparent ileal and total tract digestibility of nutrients and energy were calculated according to the following formulas (Olukosi et al., 2007):

Nutrient digestibility (%) =

$$\{1-[(AIA_{feed} / AIA_{digesta\ or\ excreta}) \times (Nutrient_{digesta\ or\ excreta} / Nutrient_{feed})]\} \times 100$$

Ileal digestible energy (**IDE**) or apparent metabolizable energy (**AME)** of the diet (kcal/kg) =

$$GE_{feed} - [GE_{digesta\ or\ excreta} \times (AIA_{feed}/AIA_{digesta\ or\ excreta})]$$

Determined dietary AME values were then used to calculate AME of test samples as described by Scott et al. (1998a). Based on the values reported in the NRC (1994), the energy contribution of the fixed ingredients making up 20% of the experimental diets in the field pea and barley studies were 725.7 and 561.3 kcal/kg, respectively.

AME of the test sample (kcal/kg) =

(AME of the experimental diet – energy of fixed ingredients of the diet) × 100/80

Dietary IDE is a measure of the amount of energy absorbed by the bird up to the ileum, minimizing the confounding effects of hindgut microbiota on energy measurement using excreta samples (Olukosi et al., 2007).

6.2.5 Statistical analyses

Data of growth performance and apparent ileal and total tract digestibility of nutrients and energy were analyzed by using the GLM procedure of SAS (SAS Institute, 2002). Means were separated using the Student-Newman-Keuls (SNK) test (Kuehl, 2000). Differences were considered significant at $P \leq 0.05$.

The regression procedure of SAS (SAS Institute, 2002) was used to investigate the relationship (R^2) between *in vivo* AME of test ingredients with

physico-chemical characteristics and growth performance variables (Fairbairn et al., 1999).

Although this was originally suggested for wheat samples (Jha et al., 2011; Chapters 4 and 5), variations in different characteristics of the test samples may also be classified into 3 groups: 1) wide variation, CV > 10%; 2) medium variation, CV from 5 to 10%, and 3) small variation, CV < 5%. This classification will help to explain our observations with respect to variations in physico-chemical characteristics of field pea and barley samples.

6.3 Results

6.3.1 Physico-chemical characteristics of field pea samples

Variation in test weight was small (CV=1.8 %) and samples 7 and 4 had the lowest (80.4 kg/hL) and highest (84.5 kg/hL) test weight, respectively. However, variation level for 1,000 kernel weight among pea samples was medium (CV= 8.3%) and it ranged from 216 g for samples 5 and 8 to 268 g for sample 1 (Table 6.2).

Variations in DM (CV= 0.1%), GE (CV=0.9%), and starch (CV= 3.6%) contents of the pea samples were small (Table 6.2). However, other chemical characteristics including ash (CV= 18.3%), ether extract (CV= 15.6%), CP (CV= 13.6%), and NDF (CV= 10.0%) varied widely. The CP content of pea samples ranged from 19.3% in sample 6 to 29.6% in sample 5. Ether extract content ranged from 0.9 % in sample 7 to 1.3% in sample 6. Samples 3 and 7 had the lowest (9.1%) and highest (11.7%) NDF content, respectively.

Concentrations of both essential and non-essential AA varied substantially among field pea samples (Table 6.3). In terms of essential AA, the lowest and highest variations were found for Thr (CV = 11.2%) and Arg (CV = 25.0%), respectively. The Met concentrations ranged from 0.19% in samples 1 and 8 to 0.27% in sample 5. Sample 6 (1.40%) and sample 3 (2.13%) had the lowest and highest Lys concentrations, respectively. The Thr content of test samples ranged from 0.66% in sample 6 to 0.92% in sample 5. For non-essential AA, the lowest and highest variations were observed in the content of Cys (CV= 7.9%) and Asp (CV = 28.7%), respectively. The Cys concentrations ranged from 0.28% in samples 3 and 8 to 0.34% in sample 4.

6.3.2 Physico-chemical characteristics of barley samples

Barley samples were either hulled or hulless and this characteristic should be taken into consideration as comparisons are made among barley samples tested in the present study. Variation level for test weight was medium (CV= 9.3 %) and samples 1(hulled) and 7(hulless) had the lowest (58.3 kg/hL) and highest (75.7 kg/hL) test weight, respectively. However, the 1,000 kernel weight varied widely (CV= 14.8%) among barley samples and it ranged from 35.8 g in sample 1(hulled) to 54.0 g in sample 5 (hulless; Table 6.4).

Variations in DM (CV= 0.4%) and GE (CV=1.0%) contents of the barley samples were small, but variation for starch concentration (CV= 6.6%) was medium (Table 6.4). However, concentrations of other chemical characteristics including pentosan (CV = 18.1%), CP (CV= 14.4%), β-glucan (CV= 12.2%), ether extract (CV= 11.5%), and ash (CV= 10.80%) varied widely. The lowest

(4.14%) and highest (5.86%) β-glucan contents were found in samples 3 (hulled) and 5(hulless), respectively. The CP content ranged from 12.8% in sample 2(hulless) to 19.6% in sample 5 (hulless). Samples 8 (hulled) and 2 (hulless) had the lowest (1.7%) and highest 2.6%) ether extract content, respectively.

Contents of both essential and non-essential AA varied considerably among barley samples (Table 6.5). In terms of essential AA, the lowest and highest variations were found for Lys (CV = 5.7%) and Phe (CV = 16.7%), respectively. The Met concentrations ranged from 0.19% in samples 2 (hulless) and 8 (hulled) to 0.29% in sample 5(hulless). Samples 7 (hulless) and 8 (hulled) (0.47%) and sample 3 (hulled; 0.55%) had the lowest and highest Lys concentrations, respectively. The Thr content of barley samples ranged from 0.38% in sample 8 (hulled) to 0.53% in sample 3(hulled). For non-essential AA, the lowest and highest variations were observed in the content of Asp (CV= 9.2%) and Glu and Tyr (CV = 18.0%), respectively. The Cys content ranged from 0.25% in sample 8 (hulled) to 0.39% in sample 5 (hulless). All experimental diets from both studies were also analyzed for nutrients. The analyzed chemical contents of the diets are presented in Tables 6.6 (field pea diets) and 6.7 (barley diets).

6.3.3 Growth performance variables

6.3.3.1 Field pea study

The BW of all groups of birds at day 7 was not significantly different. Feeding experimental diets from 8 to 13 d of age had a significant effect on BW gain, feed intake, and FCR (Table 6.8). Birds fed samples 4 and 5-based diets had the lowest and highest BW gain, respectively ($P < 0.001$). For FCR, these groups

of birds had the lowest (sample 5) and highest (sample 4) FCR as well (*P* < 0.001). Birds fed sample 5 diet had the highest feed intake whereas birds received sample 4-based diet had the lowest feed intake (*P* = 0.007). The overall mortality rate in field pea study was 0.8% and was not influenced by dietary treatment.

6.3.3.2 Barley study

The BW of all groups of birds at day 7 was not significantly different. In this experiment (Table 6.9), birds fed with samples 3 and 5-based diets had the highest and lowest BW gain, respectively (*P* =0.002). Sample 3 had the lowest and sample 5-fed birds had the highest FCR (*P*= 0.001). Feed intake was highest in group of birds fed sample 7 and lowest in sample 6-fed group (*P* = 0.042). The overall mortality rate in barley study was 2% and diets had no effect on the mortality.

6.3.4 Digestibility of nutrients and energy

6.3.4.1 Field pea study

Samples 3- and 5 - based diets had the lowest (42.8%) and highest (55.4%) apparent ileal DM digestibility, respectively (*P* < 0.001; Table 6.10). Apparent total tract digestibility of DM (*P* < 0.001) ranged from 48.2% for sample 3 diet to 61.2% for sample 5 (Table 6.11).

Birds fed with samples 6- and 5- based diets had the lowest (53.6 and highest (70.1%) apparent ileal starch digestibility, respectively (*P* < 0.001; Table 6.10). Apparent ileal digestibility of dietary GE ranged from 50.5% for sample 3 to 60.8% for sample 5-based diet (*P* < 0.001). Dietary IDE (*P* < 0.001) ranged

from 2,237 kcal/kg of DM in sample 3-based diet to 2,699 kcal/kg of DM in sample 5-based diets. In terms of the total tract GE digestibility of the diets, samples 3 and 5-based diets had the lowest (55.3%) and highest (66.2%) digestibility, respectively (P < 0.001, Table 6.11). Dietary AME (P < 0.001) ranged from 2,451 kcal/kg of DM in sample 3-based diet to 2,939 kcal/kg of DM in birds fed sample 5-based diet. The AME values of field pea samples (calculated from dietary AME) varied widely and ranged from 2,157 kcal/kg of DM for sample 3 to 2,767 kcal/kg of DM for sample 5.

Apparent ileal digestibility of dietary protein ranged from 69.2% in sample 4 to 74.9% in sample 5-based diets (P = 0.004; Table 10). For the total tract digestibility of CP (P < 0.001), sample 7 had the lowest (50.5%) and sample 1 had the highest (63.5%) values (Table 6.11). Apparent ileal digestibility of all dietary AA differed significantly among treatments (Table 6.10). Samples 7 and 3 diets had the lowest (83.5%) and highest (88.8%) ileal Met digestibility, respectively. The Lys digestibility ranged from 74.8% in sample 6-based diet to 84.1% in birds fed sample 2-based diet. A similar pattern, as seen for Lys, was observed for ileal digestibility of Thr.

6.3.4.2 Barley study

Samples 5 and 4-based diets had the lowest (45.2%) and highest (63.3%) apparent ileal DM digestibility, respectively (P < 0.001; Table 6.12). Apparent total tract digestibility of DM varied significantly and ranged from 48.6% in sample 5 to 68.7% in sample 2-based diets (Table 6.13).

Birds fed with samples 5 and 4-based diets had the lowest (70.3%) and highest (94.5%) apparent ileal starch digestibility, respectively ($P < 0.001$; Table 6.12). Apparent ileal digestibility of dietary GE differed significantly and ranged from 47.9% in sample 5 to 65.9% in sample 4-based diets. Dietary IDE ranged from 2,168 kcal/kg of DM in sample 5-based diet to 2,926 kcal/kg of DM in sample 4- based diets ($P < 0.001$). In terms of the total tract GE digestibility of the diets, samples 5 and 2-based diets had the lowest (52.0%) and highest (71.6%) digestibility, respectively ($P < 0.001$, Table 6.13). Dietary AME ($P < 0.001$) ranged from 2,351 kcal/kg of DM in sample 5-based diet to 3,130 kcal/kg of DM in birds fed sample 4-based diet (Table 6.13). The AME of barley samples (calculated from dietary AME) varied widely and ranged from 2,237 kcal/kg of DM for sample 5 to 3,211 kcal/kg of DM for sample 4.

Birds fed with samples 5 and 4-based diets had the lowest (54.9%) and highest (74.6%) apparent ileal digestibility of CP, respectively ($P = 0.004$; Table 6.12). The total tract digestibility of CP ($P < 0.001$) ranged from 39.9% for sample 5 diet to 59.5% for sample 4 diet (Table 6.13). Apparent ileal digestibility of all dietary AA varied significantly among treatments (Table 6.12). Samples 5 and 8 diets had the lowest (82.0%) and highest (90.4%) ileal Met digestibility, respectively. The Lys digestibility ranged from 75.4% in sample 5 to 84.4% in birds fed sample 4 diet. The same pattern was also observed for ileal digestibility of Thr.

The AME of field pea samples was positively related with the ileal digestibility of dietary starch ($R^2 = 0.77$, $P = 0.004$; Table 6.14). The AME was

not related with any other characteristics of field pea samples. As stated earlier, the AME value of barley sample 5 was unusually lower than other 7 barley sample (it was an outlier) and as a result, it was not included in regression equations of AME of barley samples with other characteristics. The AME was negatively related with β-glucan content of barley samples, although this relationship was not significant ($R^2 = 0.52$, $P = 0.068$). Thus, there was no relationship of AME with any characteristics of barley samples used in the present study.

6.4 Discussion

Physical characteristics of barley samples used in the present study (58.3 to 75.7 kg/hL and 38.5 to 54.0 g for test weight and 1,000 kernel weight, respectively) were within a relatively similar range as other studies. Specific weight of 426 barley samples ranged from 58.5 to 72.0 kg/hL (Metayer et al., 1993). Fairbairn et al. (1999) reported that test weight and 1,000 kernel weight of 20 barley samples collected from western Canada were 54.2 to 69.7 kg/hL and 33.2 to 43.8 g, respectively. In another study on 39 barley samples, density of samples ranged from 47.9 to 71.5 kg/hL (Zijlstra et al., 2011). However, to the authors' knowledge, there is a lack of information on physical characteristics of field peas in the literature related to poultry feeding.

As with the present study, previous studies have also reported wide variations in chemical characteristics of barley samples. The CP and starch contents of barley samples were 11.2 to 16.5% and 54.7 to 63.1%, respectively (Villamide et al., 1997). In the study of Fairbairn et al. (1999), these values were

11.8 to 13.6 and 47.1 to 49.9 %., respectively. Starch content of barley samples in the present study was similar to values reported by Villamide et al. (1997), but there was a wider range in protein content of barley samples in the present study. Overall, amino acid content of barley samples in the present study were in a similar range as reported previously on samples collected from western Canada (Bandegan et al., 2010).

The β-glucan concentrations of barley samples reported in previous studies (Villamide et al., 1997; Fairbairn et al., 1999; Al-Marzooqi et al., 2010) ranged from 3.3 to 4.4% which is lower than the range observed in the present study. The higher range of β-glucan content in the present study might be due to hulless barley samples as they generally contain more β-glucan (M. L. Swift, Personal Communication). The AME of barley samples varied widely in the present study. Variations in AME values of barley have also been reported previously (Metayer et al., 1993; Villamide et al., 1997; Scott et al., 1998 a, b). Scott et al. (1998b) reported a range of 2,800 to 3,320 kcal/kg for the AME of 14 barley cultivars collected from 7 locations over 3 years in western Canada.

The CP and starch contents of pea samples ranged from 23.0 to 28.3% and 39.7 to 43.5%, respectively (Ravindran et al., 2010) which are in similar ranges to the present study. The Met and cys concentrations of pea samples were lower than other amino acids in the present study. This pattern has also been observed in other studies, indicating that in legumes, these amino acids are generally present at deficient levels for broiler performance (Igbasan and Guenter, 1996a, Ravindran et al., 2010; Nalle et al., 2011). Overall, amino acid content of field pea

samples in the present study were in a similar range as reported previously on samples collected from western Canada (Bandegan et al., 2010). The AMEn of pea samples ranged from 1980 to 2460 kcal/kg (Igbasan and Guenter. 1996c). Nalle et al. (2011) reported a range of 2,345 to 2,575 kcal/kg in the AME value of pea samples which is in a relatively similar range as of the present study.

According to Igbasan and Guenter (1996b), in addition to differences in nutrient contents, variation in concentrations of anti-nutritional factors can also, to some extent, contribute to differences seen in published studies in the literature. Al-Marzooqi et al. (2009) suggested that variability in ileal digestibility of AA can be due to high fiber contents of barley cultivars. In another study, Wiseman et al. (2003) reported that trypsin inhibitor (**TI**) concentration of pea samples can directly influence nutrient digestibility. They reported that digestibility of AA in pea samples with low TI was higher than samples with high TI contents. Although TI levels were not measured in the field pea samples in the present study, this factor might have played a role in observed variations in digestibility of dietary AA among pea-based diets.

Svihus and Gullord (2002) reported that variations in viscosity of barley samples can play a major role in differences in nutritive value of this feedstuff for poultry. Ribeiro et al. (2011) also demonstrated that endogenous β-glucanases concentrations can vary substantially among barley samples and these enzymes may retain their activities in the digestive tract. Although endogenous β-glucanases were not measured in the barley samples in the present study, it is possible that these variations in enzyme concentrations might have also

contributed to differences observed in digestibility of nutrients and energy of barley-based diets in the present study.

There were no relationships between physical characteristics of field peas and barley samples with their respective AME values in the present study. This observation is in agreement with Zijlstra et al. (2011) who also reported a very poor relationship (R^2 = 0.14) between digestible energy content of 39 barley samples and their density. These researchers concluded that physical characteristics cannot accurately predict DE content of barley samples in pigs. In another study, Metayer et al. (1993) also reported that specific weight poorly predicts the nutritive value of barley. Villamide et al. (1997) observed no relationship between AMEn and chemical characteristics of barley samples which is in agreement with the present study. Due to the lack of information on the relationship between AME of field pea samples with physico-chemical characteristics, it is not possible to compare the results of the present study with other reports.

The nutritional value of barely sample 5 was very low compared to other 7 barley samples used in the present study. The reasons for this situation are not clear, but it might be related to the quality of this sample. One explanation is that the hull did not leave the seed as it was being harvested (i.e., it had a high adherence rate), and this might have interfered with broiler chick ability to digest this barley sample (M. L. Swift, Personal Communication). Thus, this barely sample was not included in the regression equations of the AME of barley samples with physico-chemical characteristics.

Differences in cultivar and growing conditions could have played an important role in variations in nutritive values of the test samples observed in the present study (Villamide et al., 1997; Scott et al., 1998b; Fairbairn et al., 1999; Al-Marzooqi et al., 2010). Another potential contributing factor in this regard could be differences in methodologies for determining digestibility of nutrients and energy in various studies (Nalle et al., 2011). For example, it has been indicated in previous publications (Villamide et al., 1997; Bandegan et al., 2010) that the available information on the feeding vales of these ingredients is usually obtained from studies using adult roosters and these values might not be applicable to broiler chickens. It is also important to note that the diets used in the present studies were not a typical broiler starter diet and this might have been a limiting factor with respect to performance of birds. Igbasan and Guenter (1996b) suggested that experiments which are aimed at evaluating nutrient availability might not necessarily provide optimal conditions for growth performance of birds. This statement would also apply to the present studies as they were intended to only evaluate variation in nutrient availability of barley and field peas for broiler chickens and performance variables were not the primary focus of these studies.

6.5 Conclusions

There is not much information in the literature on variations in nutrient availability of barley and field pea samples for broiler chickens in western Canada. Although the number of samples evaluated in these two experiments was not substantial, the results of the present studies showed variation in nutritive value including AME contents of field pea and barley samples. This information

can be used for further developments (e.g., creation of database) in evaluating the quality of these feedstuffs for broiler chickens.

6.6 References

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Table 6.1 Composition of diets fed to broiler chicks

Ingredients (%)	Starter diet	Experimental diets	
		Field pea	Barley
Corn	18.0	6.4	0.0
Vegetable fat	3.8	4.0	2.0
Fish Meal	3.0	2.0	4.0
Soybean meal	26.8	1.4	7.3
Wheat	43.41	0.0	0.0
Field pea	0.0	80.0	0.0
Barley	0.0	0.0	80.0
Calcium carbonate	1.32	1.40	1.21
Dicalcium phosphate	1.50	1.52	1.31
Salt	0.42	0.46	0.39
L-Lysine HCl	0.23	0	0.66
DL-Methionine	0.23	0.48	0.44
L-Threonine	0.05	0.15	0.33
Vitamin E 5000 IU/kg	0.3	0.3	0.3
Celite[1]	0.0	1.0	0.8
Chromic oxide[2]	0.0	0.0	0.3
Vitamin-mineral premix[3]	0.5	0.5	0.5
Choline chloride premix[4]	0.5	0.5	0.5
Calculated nutrient content			
DM (%)			
Protein (%)	23	21.28	16.50
Metabolizable energy (Kcal/kg)	3,079	2,720	2,710
Calcium (%)	1.1	1.1	1.07
Available P (%)	0.5	0.5	0.5

[1]Celite Corporation, World Minerals Inc., Lompoc, CA

[2] Anachemia Canada Inc. Lachine, Quebec, Canada

[3]Vitamin/mineral premix provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 ICU; vitamin E, 35 IU; menadione, 2.0 mg; D - pantothenic acid, 14 mg; riboflavin, 5.0 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18; iodine, 0.5 mg; manganese, 70 mg; copper, 8.5 mg; zinc, 80 mg; selenium, 0.1 mg; iron, 100 mg

[4]Provided 100 mg of choline per kilogram of diet

Table 6.2 Physico-chemical characteristics of field pea samples (%) - DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	CV[1] (%)
Seed color	Yellow	Green	Green	Green	Yellow	Yellow	Green	Yellow	-
Test weight (kg/hL)	83.2	80.6	83.3	84.5	82.4	83.8	80.4	82.4	1.8
1,000 kernel weight (g)	268	256	248	250	216	246	220	216	8.3
DM	90.1	89.8	90.0	90.0	90.1	90.0	89.9	89.8	0.1
CP	25.8	24.2	24.3	22.2	29.6	19.3	28.0	22.3	13.6
GE[2] (Kcal/kg)	4,415	4,312	4,354	4,329	4,399	4,299	4,349	4,333	0.9
Starch	44.2	44.8	41.9	44.6	41.2	42.1	43.4	45.5	3.6
Ether extract	1.1	1.1	1.3	1.2	0.9	1.3	0.9	1.2	15.6
NDF[3]	9.4	10.2	11.7	10.4	10.0	10.8	9.1	12.1	10.0
Ash	1.8	3.4	2.4	2.5	3.0	2.6	2.8	2.5	18.3

[1]Coefficient of variation
[2]Gross energy
[3]Neutral detergent fiber

Table 6.3 Analyzed amino acid content of field pea samples (%) - DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	CV[1] (%)
Essential									
Histidine	0.46	0.58	0.53	0.45	0.64	0.42	0.47	0.49	14.6
Threonine	0.85	0.77	0.83	0.71	0.92	0.66	0.70	0.77	11.2
Arginine	1.53	1.76	2.01	1.41	2.50	1.23	2.02	1.34	25.0
Methionine	0.19	0.24	0.20	0.26	0.27	0.26	0.21	0.19	14.8
Valine	1.24	1.44	1.53	1.09	1.31	1.04	1.26	1.05	14.5
Phenylalanine	1.12	1.33	1.47	1.00	1.27	0.97	1.18	1.01	15.3
Isoleucine	1.08	1.30	1.45	0.98	1.29	0.95	1.18	0.98	15.9
Leucine	1.71	2.05	2.25	1.50	2.01	1.46	1.86	1.51	16.5
Lysine	1.64	1.98	2.13	1.51	1.92	1.40	1.71	1.48	15.3
Non-essential									
Aspartic acid	2.81	3.38	2.91	2.31	3.12	2.06	2.50	2.68	15.8
Glutamic acid	4.31	4.84	4.39	3.51	4.74	2.98	3.86	4.06	15.3
Serine	0.78	0.94	0.90	0.71	0.95	0.66	0.78	0.80	13.0
Glycine	1.22	1.26	1.30	1.12	1.54	0.98	1.12	1.18	13.5
Alanine	1.05	1.11	1.32	1.03	1.26	0.89	1.06	0.93	13.7
Tyrosine	0.41	0.45	0.52	0.41	0.50	0.36	0.40	0.40	12.7
Cysteine	0.29	0.31	0.28	0.34	0.34	0.32	0.30	0.28	7.9

[1]Coefficient of variation

Table 6.4 Physico-chemical characteristics of barley samples (%) - DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	CV[1] (%)
	Hulled 6 row	Hulless 2 row	Hulled 6 row	Hulled 2 row	hulless 2 row	Hulled	Hulless	Hulled 6 row	
Test weight (kg/hL)	58.3	71.8	65.9	59.6	68.6	61.2	75.7	63.9	9.3
1,000 kernel weight (g)	35.8	36.6	44.9	37.0	54.0	39.0	42.5	38.5	14.8
DM	90.1	89.5	89.9	89.8	90.1	90.6	90.3	90.3	0.4
CP	16.7	12.8	16.9	17.0	19.6	14.7	16.4	12.9	14.4
GE[2] (Kcal/kg)	4,445	4,369	4,384	4,470	4,492	4,451	4,397	4,398	1.0
Starch	54.4	65.7	56.9	56.9	57.6	57.4	63.3	62.2	6.6
Ether extract	2.1	2.6	2.0	2.1	2.2	2.2	1.9	1.7	11.5
β-glucan	4. 68	4.16	4.14	4.29	5.86	4.71	5.14	4.76	12.2
Pentosan	12.02	10.03	9.83	10.38	6.74	11.53	7.99	8.94	18.1
Ash	2.4	2.1	2.6	2.4	2.0	2.1	2.0	2.3	10.8

[1]Coefficient of variation
[2]Gross energy

Table 6.5 Analyzed essential and non-essential amino acid contents of barley samples (%) - DM basis

Item	Sample 1 Hulled 6 row	Sample 2 Hulless 2 row	Sample 3 Hulled 6 row	Sample 4 Hulled 2 row	Sample 5 hulless 2 row	Sample 6 Hulled	Sample 7 Hulless	Sample 8 Hulled 6 row	CV[1] (%)
Essential									
Histidine	0.32	0.26	0.34	0.29	0.33	0.33	0.27	0.25	11.9
Threonine	0.45	0.40	0.53	0.46	0.48	0.42	0.41	0.38	11.1
Arginine	0.62	0.56	0.71	0.62	0.66	0.59	0.64	0.52	9.6
Methionine	0.23	0.19	0.26	0.28	0.29	0.22	0.23	0.19	16.0
Valine	0.87	0.72	0.99	0.82	1.01	0.82	0.94	0.71	13.3
Phenylalanine	0.85	0.64	0.98	0.84	1.03	0.79	0.83	0.65	16.7
Isoleucine	0.64	0.47	0.71	0.61	0.72	0.57	0.61	0.48	15.5
Leucine	1.19	0.90	1.24	1.13	1.28	1.07	1.11	0.92	12.5
Lysine	0.54	0.50	0.55	0.50	0.51	0.50	0.47	0.47	5.7
Non-essential									
Aspartic acid	0.78	0.77	0.99	0.85	0.89	0.81	0.79	0.77	9.2
Glutamic acid	4.03	2.87	4.70	3.93	5.19	3.95	4.22	3.32	18.0
Serine	0.47	0.38	0.56	0.48	0.50	0.49	0.47	0.40	12.1
Glycine	0.65	0.60	0.77	0.65	0.65	0.62	0.57	0.58	9.9
Alanine	0.60	0.54	0.70	0.62	0.65	0.56	0.62	0.53	9.6
Tyrosine	0.30	0.20	0.28	0.27	0.27	0.26	0.36	0.22	18.0
Cysteine	0.29	0.27	0.32	0.35	0.39	0.27	0.30	0.25	15.3

[1]Coefficient of variation

Table 6.6 Chemical analyses of field pea-based experimental diets (%) -DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DM	89.5	89.4	89.2	89.9	90.8	90.0	89.0	90.1
GE [1](kcal/kg)	4,500	4,397	4,433	4,447	4,442	4,397	4,516	4,435
Starch	36.9	40.5	39.9	40.3	38.7	37.6	37.9	37.3
CP	23.7	22.1	23.4	21.1	26.4	20.6	25.5	21.0
Essential amino acids								
Histidine	0.39	0.43	0.37	0.35	0.47	0.29	0.41	0.38
Threonine	0.90	0.94	0.83	0.82	0.98	0.60	0.84	0.89
Arginine	1.43	1.59	1.37	1.23	2.22	0.90	1.78	1.27
Methionine	0.67	0.60	0.73	0.66	0.60	0.57	0.58	0.63
Valine	1.15	1.22	1.08	1.07	1.36	0.76	1.24	1.06
Phenylalanine	1.07	1.06	1.01	0.95	1.21	0.71	1.14	0.94
Isoleucine	1.03	1.07	0.95	0.92	1.16	0.70	1.11	0.92
Leucine	1.69	1.72	1.53	1.49	1.88	1.12	1.84	1.46
Lysine	1.63	1.62	1.45	1.44	1.71	1.09	1.69	1.41
Non-essential amino acids								
Aspartic acid	2.46	2.38	2.24	2.14	2.92	1.65	2.51	2.23
Glutamic acid	3.69	3.79	3.45	3.27	4.53	2.48	3.98	3.42
Serine	0.76	0.82	0.75	0.70	0.95	0.55	0.80	0.75
Glycine	1.14	1.24	1.00	1.01	1.24	0.77	1.06	1.14
Alanine	1.03	1.10	0.98	0.92	1.18	0.71	1.00	0.92
Tyrosine	0.41	0.42	0.42	0.40	0.53	0.28	0.38	0.35
Cysteine	0.28	0.25	0.27	0.27	0.25	0.28	0.25	0.24

[1]Gross energy

Table 6.7 Chemical analyses of barley- based experimental diets (%)-DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
	Hulled 6 row	Hulless 2 row	Hulled 6 row	Hulled 2 row	hulless 2 row	Hulled	Hulless	Hulled 6 row
DM	89.3	90.1	89.3	90.3	89.3	90.1	90.5	89.8
GE [1](kcal/kg)	4,444	4,362	4,482	4,443	4,522	4,475	4,447	4,410
Starch	40.9	47.2	43.2	42.2	43.7	43.0	45.7	45.4
CP	22.9	19.3	22.1	22.8	24.2	21.1	22.9	18.8
Essential Amino Acids								
Histidine	0.32	0.33	0.30	0.43	0.33	0.43	0.43	0.26
Threonine	1.02	0.92	0.86	1.10	1.00	0.93	0.97	0.84
Arginine	1.01	0.90	0.88	1.02	1.01	0.93	0.94	0.83
Methionine	0.77	0.74	0.71	0.78	0.87	0.81	0.80	0.84
Valine	1.10	0.94	1.01	1.31	1.09	1.09	1.24	0.83
Phenylalanine	1.03	0.88	0.96	1.18	1.16	0.99	1.10	0.81
Isoleucine	0.86	0.82	0.80	0.99	0.95	0.85	0.84	0.68
Leucine	1.51	1.41	1.35	1.66	1.59	1.47	1.40	1.15
Lysine	1.55	1.68	1.36	1.62	1.51	1.59	1.49	1.34
Non-essential Amino Acids								
Aspartic acid	1.47	1.25	1.38	1.75	1.42	1.55	1.56	1.28
Glutamic acid	4.73	3.27	4.38	4.86	5.16	4.70	4.99	3.55
Serine	0.76	0.60	0.65	0.79	0.68	0.70	0.69	0.57
Glycine	0.97	0.91	0.89	1.14	0.99	0.94	0.97	0.82
Alanine	0.88	0.80	0.84	0.98	0.86	0.79	0.86	0.76
Tyrosine	0.47	0.28	0.31	0.37	0.33	0.35	0.41	0.29
Cysteine	0.28	0.30	0.32	0.37	0.39	0.29	0.33	0.28

[1]Gross energy

Table 6.8 Growth performance variables of broiler chicks fed field peas-based experimental diets[1]

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	*Pooled SEM*	*P*-value
BW (g/bird/d)	23.0[a]	21.9[a]	18.9[b]	13.7[c]	24.3[a]	16.7[b]	19.4[b]	18.8[b]	0.83	<0.001
FI (g/bird/d)	32.7[ab]	34.9[a]	31.6[ab]	28.8[b]	33.6[a]	31.3[ab]	32.5[ab]	32.7[ab]	1.00	0.007
FCR (g feed: g gain)	1.43[d]	1.59[cd]	1.68[bc]	2.11[a]	1.38[d]	1.89[b]	1.74[bc]	1.75[bc]	0.06	<0.001

[1]All means are average of 8 cages per treatment

[a-c]Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.9 Growth performance variables of broiler chicks fed barley-based experimental diets[1]

Item	Sample 1 Hulled 6 row	Sample 2 Hulless 2 row	Sample 3 Hulled 6 row	Sample 4 Hulled 2 row	Sample 5 hulless 2 row	Sample 6 Hulled	Sample 7 Hulless	Sample 8 Hulled 6 row	*Pooled SEM*	*P*-value
BW (g/bird/d)	20.1[ab]	16.5[b]	22.8[a]	18.9[ab]	14.8[b]	15.9[b]	19.0[ab]	16.4[b]	1.34	0.002
FI (g/bird/d)	28.8[ab]	31.0[ab]	30.2[ab]	30.7[ab]	29.9[ab]	27.6[b]	31.5[a]	30.3[ab]	0.84	0.042
FCR (g feed: g gain)	1.56[bc]	1.88[ab]	1.43[c]	1.63[bc]	2.01[a]	1.73[ab]	1.66[bc]	1.86[ab]	0.08	0.001

[1]All means are average of 8 cages per treatment

[a-c] Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.10 Apparent ileal digestibility of nutrients and energy of field pea-based diets (%) [1]- DM basis

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	*Pooled SEM*	*P*-value
DM	51.3^{b}	51.4^{b}	42.8^{d}	46.4^{cd}	55.4^{a}	45.1^{d}	44.1^{d}	48.5^{bc}	0.97	<0.001
Starch	63.9^{b}	63.8^{b}	56.9^{cd}	60.7^{bc}	70.1^{a}	53.6^{d}	54.3^{d}	57.6^{cd}	1.12	<0.001
GE[2]	59.0^{a}	57.6^{a}	50.5^{c}	53.3^{bc}	60.8^{a}	52.7^{bc}	52.7^{bc}	54.7^{b}	0.96	<0.001
IDE[3] (kcal/kg)	2,655^{a}	2,533^{b}	2,237^{d}	2,370^{cd}	2,699^{a}	2,315^{cd}	2,378^{cd}	2,427^{bc}	43	<0.001
CP	73.3^{ab}	74.3^{a}	70.6^{ab}	69.2^{b}	74.9^{a}	71.5^{ab}	70.4^{ab}	72.0^{ab}	1.07	0.004
Essential amino acids										
Histidine	78.5^{ab}	84.4^{a}	77.2^{b}	76.7^{b}	79.2^{ab}	75.0^{b}	73.2^{b}	80.0^{ab}	1.69	0.001
Threonine	72.6^{bc}	77.4^{a}	69.4^{cd}	69.1^{cd}	73.7^{abc}	60.8^{e}	65.2^{d}	75.0^{ab}	1.32	<0.001
Arginine	82.3^{b}	86.8^{a}	80.4^{b}	79.7^{b}	87.7^{a}	76.0^{c}	81.6^{b}	82.3^{b}	0.93	<0.001
Methionine	88.1^{a}	88.4^{a}	88.8^{a}	87.0^{a}	85.5^{ab}	86.4^{ab}	83.5^{b}	88.6^{a}	0.88	0.001
Valine	70.0^{abc}	74.5^{a}	65.8^{c}	65.2^{c}	72.7^{ab}	56.8^{d}	65.5^{c}	67.9^{bc}	1.47	<0.001
Phenylalanine	74.1^{ab}	76.4^{a}	70.6^{b}	68.9^{b}	75.9^{a}	63.1^{c}	69.3^{b}	70.1^{b}	1.42	<0.001
Isoleucine	72.5^{ab}	76.3^{a}	68.0^{bc}	66.1^{c}	74.2^{ab}	60.7^{d}	68.1^{bc}	69.7^{bc}	1.57	<0.001
Leucine	74.2^{abc}	77.6^{a}	68.7^{cd}	68.6^{cd}	75.4^{ab}	63.5^{d}	70.0^{bc}	70.8^{bc}	1.54	<0.001
Lysine	81.5^{ab}	84.1^{a}	77.9^{bc}	78.2^{bc}	81.8^{ab}	74.8^{c}	77.2^{bc}	79.0^{bc}	1.24	<0.001
Non-essential amino acids										
Aspartic acid	75.2^{ab}	78.8^{a}	70.7^{c}	71.5^{bc}	78.4^{a}	66.8^{c}	70.4^{c}	75.0^{ab}	1.13	<0.001
Glutamic acid	80.7^{bc}	84.6^{a}	77.9^{cd}	78.5^{c}	83.5^{ab}	74.8^{d}	77.5^{cd}	81.1^{bc}	0.97	<0.001
Serine	71.1^{b}	76.4^{a}	67.3^{c}	66.7^{cd}	74.3^{ab}	62.6^{d}	66.1^{cd}	73.2^{ab}	1.24	<0.001
Glycine	74.8^{bc}	80.1^{a}	71.9^{cd}	72.0^{cd}	75.7^{bc}	65.4^{e}	68.2^{de}	77.5^{ab}	1.28	<0.001
Alanine	74.0^{bc}	78.8^{a}	70.6^{cd}	70.0^{cd}	76.7^{ab}	64.9^{e}	67.6^{de}	72.7^{bc}	1.30	<0.001
Tyrosine	68.2^{abc}	72.0^{ab}	64.1^{c}	65.5^{c}	73.7^{a}	57.6^{d}	55.8^{d}	67.4^{bc}	1.67	<0.001
Cysteine	57.5^{a}	56.7^{a}	56.8^{a}	53.0^{a}	46.4^{b}	58.3^{a}	47.8^{b}	56.0^{a}	1.62	<0.001

[1]All means are average of 8 cages per treatment

[2]Gross energy

[3]Ileal digestible energy

[a-e]Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.11 Apparent total tract digestibility of nutrient and energy of field pea-based diets (%) - DM basis[1]

Item	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	*Pooled SEM*	*P*-value
DM	57.6^{b}	57.2^{b}	48.0^{d}	54.1^{bc}	61.2^{a}	52.4^{c}	48.4^{d}	54.6^{bc}	0.98	<0.001
CP	63.5^{a}	61.3^{ab}	59.3^{abc}	56.0^{c}	57.5^{bc}	59.6^{abc}	50.5^{d}	61.8^{ab}	1.28	<0.001
GE[2]	64.0^{ab}	62.9^{bc}	55.3^{f}	61.0^{cd}	66.2^{a}	58.8^{de}	56.9^{ef}	60.7^{cd}	0.81	<0.001
Dietary AME[2] (kcal/kg)	$2,878^{a}$	$2,767^{b}$	$2,451^{d}$	$2,713^{b}$	$2,939^{a}$	$2,585^{c}$	$2,569^{c}$	$2,699^{b}$	36	<0.001
Field pea AME (kcal/kg)	2,690	2,552	2,157	2,484	2,767	2,324	2,304	2,467	-	-

[1]All means are average of 8 cages per treatment

[2]Gross energy

[3]Apparent metabolizable energy

[a-f]Means within a column with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.12 Apparent ileal digestibility of nutrients and energy of barley-based diets (%) [1]- DM basis

Item	Sample 1 Hulled 6 row	Sample 2 Hulless 2 row	Sample 3 Hulled 6 row	Sample 4 Hulled 2 row	Sample 5 hulless 2 row	Sample 6 Hulled	Sample 7 Hulless	Sample 8 Hulled 6 row	*Pooled SEM*	*P*-value
DM	57.5[bc]	62.3[a]	59.6[b]	63.3[a]	45.2[d]	55.5[c]	57.6[bc]	58.3[bc]	0.81	<0.001
Starch	90.0[ab]	87.7[b]	93.7[a]	94.5[a]	70.3[c]	93.2[a]	85.9[b]	87.1[b]	1.56	<0.001
GE[2]	60.5[bc]	64.1[ab]	62.2[b]	65.9[a]	47.9[d]	58.2[c]	60.3[bc]	61.2[bc]	0.97	<0.001
IDE[3] (kcal/kg)	2,688[bc]	2,795[ab]	2,788[ab]	2,926[a]	2,168[d]	2,607[c]	2,680[bc]	2,698[bc]	43	<0.001
CP	71.5[ab]	66.4[bc]	66.4[bc]	74.6[a]	54.9[d]	62.9[c]	67.9[bc]	67.1[bc]	1.54	<0.001
Essential amino acids										
Histidine	74.8[a]	75.5[a]	71.8[a]	83.2[a]	59.0[b]	78.1[a]	81.7[a]	69.8[a]	3.39	0.002
Threonine	76.2[ab]	77.2[ab]	73.1[ab]	81.1[a]	67.8[b]	69.2[b]	76.3[ab]	73.9[ab]	2.60	0.013
Arginine	77.8[a]	75.8[ab]	71.5[ab]	80.6[a]	64.2[b]	68.9[ab]	75.1[ab]	74.4[ab]	3.00	0.010
Methionine	87.1[abc]	87.2[abc]	85.4[c]	90.1[ab]	82.0[d]	85.9[bc]	87.4[abc]	90.4[a]	1.06	<0.001
Valine	74.2[ab]	69.0[ab]	68.4[ab]	78.9[a]	55.4[c]	64.9[bc]	74.9[ab]	64.8[bc]	3.19	0.001
Phenylalanine	78.4[a]	74.0[ab]	75.4[a]	82.1[a]	64.2[b]	70.8[ab]	77.4[a]	72.8[ab]	2.69	0.001
Isoleucine	74.9[ab]	72.3[ab]	68.7[ab]	78.2[a]	61.3[b]	65.4[ab]	72.6[ab]	67.1[ab]	3.22	0.012
Leucine	78.0[a]	74.0[ab]	70.9[ab]	79.3[a]	62.5[b]	68.3[ab]	73.5[ab]	68.8[ab]	3.07	0.007
Lysine	84.0[a]	84.2[a]	78.1[abc]	84.4[a]	75.4[c]	76.9[bc]	83.3[ab]	81.3[abc]	2.19	0.014
Non-essential amino acids										
Aspartic acid	66.3[ab]	62.4[ab]	61.8[ab]	73.3[a]	50.1[b]	58.7[ab]	67.8[ab]	64.9[ab]	4.09	0.015
Glutamic acid	84.4[a]	77.7[a]	83.2[a]	86.8[a]	67.0[b]	80.4[a]	81.9[a]	80.8[a]	2.30	<0.001
Serine	74.4[a]	69.5[a]	67.7[a]	76.6[a]	54.6[b]	62.8[ab]	71.4[a]	68.8[a]	3.52	0.002
Glycine	67.7[ab]	69.3[ab]	65.2[ab]	75.9[a]	56.1[b]	61.2[ab]	69.7[ab]	66.3[ab]	3.61	0.017
Alanine	71.3[ab]	68.9[abc]	66.4[abc]	76.1[a]	55.5[c]	57.9[bc]	70.7[ab]	67.5[abc]	3.60	0.002
Tyrosine	76.1[a]	61.1[ab]	60.8[ab]	72.0[a]	47.5[b]	59.6[ab]	71.9[a]	62.2[ab]	4.10	0.003
Cysteine	55.3[c]	64.2[b]	62.3[b]	70.8[a]	49.9[c]	53.9[c]	61.3[b]	62.0[b]	1.81	<0.001

[1]All means are average of 8 cages per treatment
[2]Gross energy
[3]Ileal digestible energy
[a-c]Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.13 Apparent total tract digestibility of nutrient and energy of barley-based diets (%) - DM basis[1]

Treatments	Sample 1 Hulled 6 row	Sample 2 Hulless 2 row	Sample 3 Hulled 6 row	Sample 4 Hulled 2 row	Sample 5 hulless 2 row	Sample 6 Hulled	Sample 7 Hulless	Sample 8 Hulled 6 row	*Pooled SEM*	*P*-value
DM	62.2[cd]	68.7[a]	63.6[cd]	66.1[b]	48.6[e]	61.4[d]	64.2[bc]	62.8[cd]	0.67	<0.001
CP	56.3[ab]	55.7[ab]	56.7[ab]	59.5[a]	39.9[c]	52.9[b]	56.1[ab]	52.7[b]	1.12	<0.001
GE[2]	66.8[cd]	71.6[a]	68.7[bc]	70.4[ab]	52.0[e]	65.7[d]	67.8[cd]	66.4[cd]	0.7	<0.001
Dietary AME[3] (kcal/kg)	2,970[c]	3,121[a]	3,079[ab]	3,130[a]	2,351[d]	2,942[c]	3,013[bc]	2,930[c]	31	<0.001
Barley AME (kcal/kg)	3,011	3,200	3,147	3,211	2,237	2,976	3,065	2,961	-	-

[1]All means are average of 8 cages per treatment

[2]Gross energy

[3]Apparent metabolizable energy

[a-d] Means within a row with no common superscripts differ significantly ($P \leq 0.05$)

Table 6.14 Relationships of *in vivo* AME[1] of field pea and barely samples with physico-chemical characteristics and growth performance variables

Item	Field pea samples		Barley samples[2]	
	R^2	*P*-value	R^2	*P*-value
Test weight	0.02	0.916	0.06	0.601
1,000 kernel weight	0.03	0.967	0.001	0.950
GE[3]	0.31	0.155	0.03	0.729
Starch	0.02	0.757	0.03	0.729
Ether extract	0.28	0.174	0.33	0.176
CP	0.17	0.305	0.05	0.632
NDF[4]	0.17	0.306	-	-
β-glucan	-	-	0.52	0.068
Pentosan	-	-	0.01	0.805
Ileal digestibility of dietary starch	0.77	0.004	0.08	0.532
Ash	0.01	0.943	0.02	0.735
BW gain	0.36	0.119	0.10	0.486
Feed intake	0.15	0.347	0.34	0.167
FCR[5]	0.30	0.159	0.03	0.713

[1]Apparent metabolizable energy
[2]Barley sample 5 was not included in regression equations for AME value of barley samples with physico-chemical characteristics
[3]Gross energy
[4]Neutral detergent fiber
[5]Feed conversion ratio

Chapter 7

General discussion

Feed is considered as the most expensive component of the total production cost (Leeson 2004; Zijlstra and Beltranena, 2007). Volatility in feed ingredient prices, particularly over the past few years, has been very challenging for the industry. Expansion of the biofuel industry and increasing demand for human foods are amongst the most important contributing factors to the current upward trend in feed costs (Best 2009; Patience et al., 2009).

It is becoming much more important to use pragmatic approaches to alleviate some of the negative impacts that these challenges can have on the poultry industry (Yegani et al., 2011). The overall objective of this PhD thesis was divided into three mains areas: Evaluation of variations in nutrient availability of feed ingredients commonly used in broiler chicken rations; using exogenous enzymes to reduce these variations, and most importantly, prediction of variations in nutrient availability of feed ingredients for broilers.

Considering the limitations associated with the supply of feedstuffs on a global basis, it is very important for the industry to be able to obtain the greatest proportion of nutrients out of the feedstuffs. A critical step in this process is to know the actual digestible nutrients available to the animal from feed ingredients such as wheat, corn, barley, and field peas so that the diet formulation can meet the animal's requirements more closely (Leeson, 1997; Yegani et al., 2011).

The nutrient content of feedstuffs is normally governed by genetics (cultivar) and environment (Scott et al., 1998). Feedstuffs are often obtained from different geographical locations and as a result, their nutrient content can vary substantially (van Kempen and Simmins,

1997). The *in vivo* digestibility trials reported in this thesis (Chapters 2, 3, 4, 5 and 6) showed the existence of variations in availability of nutrients and energy of tested feedstuffs in broiler chickens. However, the extent of these variations was not the same for all the ingredients. Corn samples were less variable compared to wheat, barley, and field pea samples.

Data presented in these chapters can be used for further developments in the area of quality evaluation of these feed ingredients for broiler chickens as these studies were part of a larger feed quality evaluation program in the province of Alberta (Feed Quality Evaluation/NIRS ACIDF Project. 2007). It is important to note that testing several samples of each variety (e.g., wheat) from multiple locations and crop years could have probably provided a stronger basis for evaluating variations in nutrient availability (M. L. Swift, Personal Communication) as this information can better reflect the situation encountered in the feed industry for diet formulation.

It is not only needed to have a good understanding of these variations, but it is also important to use practical approaches in order to reduce these variations as much as possible. Using exogenous enzymes is one of the strategies in this regard (Bedford et al., 1998; Svihus and Gullord, 2002; Cowieson, 2005). Inclusion of different exogenous enzymes (xylanase; xylanase, amylase, and protease; xylanase and β-glucanase) in corn-soy diets was, in some cases, associated with transient effects on the ileal digestible energy and ileal amino acid digestibility of some of the diets, although enzyme treatments had no effects on performance variables. The responses to enzyme supplementation varied depending on corn source, enzyme product type, and age of the birds. This indicates that enzyme supplementation may not always be associated with positive effects on performance and digestibility of nutrient and energy (Cowieson et al., 2006).

Although negative control diets were formulated to be deficient in metabolizable energy (compared to positive control diets), analyzed values showed that in some cases, there were no differences between negative and positive control diets and this might have a been limiting factor for accurate evaluation of the efficacy of exogenous enzymes used in this study (Chapters 2 and 3). As discussed in Chapter 2, it is also important to get a better understanding of enzyme-substrate specificity, the gut microbiota, and immune system activity as these entities can all be of significant help to increase our knowledge of the effects of exogenous enzymes in corn-soy diets (Choct, 2006).

It was also observed that supplementing wheat- and triticale-soy diets with a mixture of xylanase, amylase, and protease increased the AME value of wheat and triticale samples, however, the exogenous enzyme product had small impact on reducing variations in AME value among the test samples. This was likely due to the narrow range that existed in AME value of the wheat and triticale samples. One of the factors that can influence responses to enzyme supplementation is the nutritional quality of feedstuffs as exogenous enzymes elicit greater positive effects when the quality of the ingredient is poorer (Wyatt et al., 1999; Cowieson, 2010). This may explain the difference in magnitude of responses to enzyme supplementation among the samples (Chapter 5). Selecting test samples with wide variations in quality characteristics (Regmi et al., 2008; Zijlstra et al., 2011) might have provided a better opportunity for evaluating the effect of the enzyme product in reducing variations in AME values.

Another important aspect of the research projects presented in this thesis was to achieve the ability to predict these variations which will allow a more accurate diet formulation. Increasing the accuracy of diet formulation should improve animal production performance and reduce nutrient excretion due to over-formulation (Scott 1996; van Kempen and Simmins 1997;

Patience et al. 2009; Yegani et al., 2011). There are a few approaches that can be taken when it comes to the prediction of nutritive value of feed ingredients (Carre 1991; Leeson 1997; van Kempen and Simmins 1997; Hughes and Choct 1999; Losada et al. 2009, 2010; Yegani et al., 2011).

Physical measurements such as test weight and 1,000 kernel weight have been used to assess feed quality. However, in general, these measurements are not good indicators of digestible nutrient content (Zijlstra et al., 1999; 2011). Our observations (Chapters 4, 5, and 6) were also in agreement with this pattern as no relationships were found between AME of test samples and their physical characteristics.

Analyzing samples in the laboratory can certainly provide good information on the nutrient or proximate content (e.g., protein, fat, fiber) of a feedstuff or ration, but these analyses are time-consuming and expensive and as a result, cannot be of direct help when immediate answers are required (Leeson, 1997; Fairbairn et al., 1999; Zijlstra, 2006; Noblet and Jaguelin-Peyraud, 2007; Losada et.al., 2009; 2010; Yegani et al., 2011). In addition, these analyses do not provide information on the digestibility of the nutrients (van Barneveld, 1999; Boisen, 2000). This is in line with our observations as well. For example, there was no relationship between AME of wheat and triticale samples and their chemical characteristics (Chapter 5). A similar pattern was also seen in the barley study (Chapter 6). This situation warrants the use of other approaches that can better reflect the actual expected responses in the animal.

As stated in the beginning of this chapter as well, the digestibility of nutrients and energy in a feedstuff can also be determined by feeding animals in an *in-vivo* study (i.e., animal trial). We measured the difference in nutrient content fed to the animal with that excreted by the animal. The difference is assumed to have been digested. An *in-vivo* experiment is the most

accurate approach, but it is a long, labour-intensive, and expensive procedure and, therefore, has very limited applications for routine feed quality evaluation. A faster and less expensive approach is to simulate the digestive system of the animal under *in vitro* conditions. *In vitro* techniques need to be validated using the *in-vivo* assay to ensure that there is a solid relationship between the two methods. Once the *in vitro* method is validated, it provides the opportunity to analyze large quantities of samples relatively inexpensively (Yegani et al., 2011; Yegani and Korver, 2012).

The *in vitro* digestibility method used was able to accurately predict AME of tested wheat and triticale samples for broiler chicks (Chapter 4). This method was validated using a modified broiler chick bioassay testing the same samples. Previous in vitro methods (Valdes and Leeson, 1992; Losada et al., 2009) were validated with in vivo data from adult roosters whereas the current in vitro assay was validated with AME values determined in broiler chicks. In addition, the current in vitro method was specifically tested with wheat and triticale samples as opposed to other in vitro techniques (Valdes and Leeson, 1992; Losada et al., 2009) that used multiple ingredients or diets to develop prediction equations.

In addition, as suggested by published literature in this area of research, chemical characteristics were also added into the equation to increase the accuracy of prediction of *in vivo* AME of the 8 test samples (Chapter 4). It is hoped that the current in vitro method will help to develop near infrared reflectance spectroscopy (NIRS) calibration equations to predict AME of wheat samples used in the poultry industry. Developing calibration equations with the data generated from the in vitro digestibility technique has been already started (M. L. Swift, Personal Communication).

In spite of this development, it must be noted that the test samples used might have not necessarily represented wheat of different variety from different locations and various crop years and this could have been a limiting factor. Thus, testing more wheat samples from different sources using the current in vitro assay will be of help to expand the database required for NIRS calibration equations. It is also of benefit to the feed industry if in vitro digestibility methods can be developed to predict AME of barley and field pea samples for broiler chickens. This can be important as increasing trend in feed cost may necessitate the use of barley and field pea. In vitro techniques specific to these feedstuffs can provide data for NIRS technology which can be of benefit to the feed industry where barley and field peas are used in diets. Considering that exogenous enzymes are commonly used in the poultry industry, it would be of interest to the industry if the *in vitro* assays could also predict response of enzymes on nutritive value of cereal grains (Bedford and Classen, 1993). The results showed that the in vitro method was not able to predict response of a mixture of xylanase, amylase, and protease on AME value of wheat and triticale samples. Although it is not clear as to why this approach was not successful, it is likely that the in vitro environment did not provide optimal conditions required for the exogenous enzyme product to work (Chapter 5).

In conclusion, our observations showed that variations exist in nutritive values of wheat, barley, and field peas. The information presented in this thesis particularly on the in vitro digestibility technique may be used for further developments in the evaluation of nutritional quality of feed ingredients for broiler flocks in western Canada. This is of particular importance with respect to developing NIRS calibration equations for predicting AME of wheat samples for broiler chickens.

7.1 Future directions

The NIRS technology will likely become more available to the animal feed industry (Patience et al., 2009). By using this technology, the nutrient content of different feedstuffs can be predicted quickly (usually within a few minutes). “Real-time” analysis supports real-time decision making for both the sellers as well as the buyers of the feedstuffs. In other words, payment for a load of feedstuff arriving at the feed mill could be based on the actual feeding value of that specific load (Scott, 1996; Leeson, 1997; Feed Quality Evaluation/NIRS ACIDF Project, 2007; Yegani et al., 2011).

As previously reviewed (Yegani et al., 2011; Yegani and Korver, 2012), the NIRS technology needs to be specifically calibrated to estimate nutrient content or digestibility of different feedstuffs such as wheat, corn, barley, and field peas for broilers. Developing the calibration models relies on a reference method such as the *in-vivo* or *in-vitro* techniques that are described in Chapters 4 to 6 of this thesis. Creation and up-dating of the calibration databases are very important and the *in vitro* digestibility technique described for wheat and triticale samples can generate data for NIRS to predict AME value of cereal samples for poultry. This ability can be of significant benefit to the poultry industry.

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