EVALUATION OF VACUUM POST-PELLET APPLICATIONS OF BIOACTIVES TO BROILER FEED ON EFFICACY AND PROTECTED DELIVERY

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ABSTRACT

The use of vacuum coating is mostly limited to production of high fat containing extruded aqua and pet diets. The physical characteristics of extrudates are favourable for vacuum coating due to their high porosity and durability. However, with pelleted feed for broilers, there are potentially several opportunities, but there are also challenges; these are explored here. The opportunities identified were inclusion of high level of oils, protected delivery of feed additives (e.g., enzymes, probiotics, vaccines, etc.), improved and safe use of offensive feed additives and improvement of shelf-life of feed and additives. Challenges include the relatively high density of pellets (low porosity) which limits liquid infusion, increased processing cost and decreased feed throughput. However, feed ingredients selection and alternating processing variables (temperature, moisture, die specifications etc.) were deemed to overcome the challenges of low porosity. Three experiments were conducted to evaluate the use of vacuum coating in pelleted feed. In the first experiment, the effect of particle size on post-pellet oil absorption (OA), porosity, pellet durability index (PDI) and bulk density were investigated. The three particle sizes for three grains (wheat, barley and corn) were pelleted using a 4.7 mm die to get whole grain (WP), coarse (CP), and fine (FP) grind pellets. The pellets were coated with 15% canola oil without (VC-) and with (VC+; 0.3 bar) vacuum coating. The grain type was found to have a significant effect on the particle size when ground through either fine (3.2 mm) or coarse (6.4 mm) screen. With coarse grinding, the mean particle size was 1896, 1290 and 1057 µm, respectively for barley, wheat and corn; with fine grinding, the mean particle size was 1153, 767 and 732 µm, respectively. Porosity of CP from wheat and corn was significantly ($P<0.01$) higher than WP and FP. For barley, there was no difference in porosity of CP and FP but both were significantly higher than WP. For wheat, OA of CP was highest ($P<0.01$), but no significant

difference was found between FP and WP. However, for barley, higher OA was found in FP followed by CP and WP. In corn, OA of CP was higher than for FP or WP. Vacuum coating (VC+) improved ($P<0.01$) OA of all pellets compared to VC-. Porosity was positively correlated with OA and negatively correlated to PDI and bulk density. Overall, the first experiment suggested that alteration of particle size and grain type could be the options for improving the oil absorption by vacuum coating. A second experiment was conducted to observe the effect of enzyme addition method (EAM; E-, without enzyme; PreE+, Pre-pellet addition of enzyme; PosE+, post-pellet addition of enzyme), conditioning temperature (CT; 65 or 95°C) and coating method (CM; VC- or VC+) on broiler performance when fed wheat-rye-based diets. Enzyme addition (pre or post-pellet addition in comparison to without enzyme) significantly improved ($P<0.01$) the body weight at 21 and 35d. Higher CT (95°C) improved feed conversion ratio (FCR) in both starter ($P<0.01$) and grow/finish phase ($P=0.04$) and PDI of both starter and grow/finish pellets ($P<0.01$) when compared to low CT (65°C). Vacuum coating did not have any effect on the diet extract viscosity, animal performance or digesta viscosity in either of the phases. However, with post-pellet vacuum coating, there was high retention of xylanase activity after processing. Vacuum coating significantly ($P<0.05$) reduced the relative length of small intestine of broilers at 21d but not at 35d. In the third experiment, broiler grow/finish diets were stored in an incubator (37°C) to see if vacuum coating can improve the shelf-life of feed. The results showed post-pellet vacuum-coated pellets retained higher enzyme activity after 15 days of storage. Although no effect of vacuum coating on animal performance was observed, vacuum coating was able to protect the enzyme during processing and storage. Further work needs to be done to translate these benefits to improve animal performance, which might be achieved using various vacuum coating and processing conditions, and bioactives.

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DEDICATION

I want to dedicate this work to my father (late Tika Ram Lamichhane) and mother (Laxmi Lamichhane), who struggled all their life against all odds to bring me up and to help me obtain higher education. Even though, my father is not with me, his inspiration is always with me to pursue my dreams.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
µm	Micrometer
ABW	Average body weight
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy (nitrogen corrected)
aw	Water activity
BWG	Body weight gain
CM	Coating method
cP	Centipoise
CP	Coarse grind pellets
CT	Conditioning temperature
CV	Coefficient of variation
d	Day
DDGS	Distiller's dried grains with solubles
Dig.Visc	Digesta viscosity
Duo	Duodenum
E-	Without enzyme
EAM	Enzyme addition method
FCR	Feed conversion ratio
FP	Fine grind pellets
g/b/d	Gram/bird/day
g/cm/s	Gram/centimeter/second
GIT	Gastrointestinal tract
IU	International unit
Jej	Jejunum
kcal	Kilocalorie
kg	Kilogram
kPa	Kilopascal
lb	Pound

ME	Metabolisable energy
MPa	Megapascal
MPDI	Modified pellet durability index
mU	milliunit
N	Normal
NIR	Near Infrared reflectance
NR	Nitrogen retention
NS	Not-significant
NSP	Non-starch polysaccharides
OA	Oil absorption
PDI	Pellet durability index
PosE+	Post-pellet addition of enzyme
PPLA	Post-pellet liquid application
PreE+	Pre-pellet addition of enzyme
Prov	Proventriculus
rpm	Revolutions per minute
SEM	Standard error of means
VC-	Without vacuum
VC+	With vacuum
WP	Whole grain pellets

1.0 INTRODUCTION

Pre-pellet delivery of high (>5%) levels of oil reduces pellet durability and results in losses in bird performance and customer satisfaction. Similarly, pre-pellet addition of bioactives could result in losses in activity when the conditioning temperature is high. Post-pellet application (i.e., spraying) of oil or bioactives may contribute to heterogeneous distribution between intact pellets and fines. Then when the fines are returned to the pelleter the bioactive is destroyed. Post-pellet application of fats and heat-sensitive bioactives can also be achieved through vacuum coating. Post-pellet vacuum coating can improve the storage life and bioavailability of bioactives sensitive to oxidation and/or digestion. As for encapsulation, vacuum coating may also offer a means of improving delivery of noxious compounds and improve safety of handling these compounds. One of the challenges is to improve our understanding of how to achieve higher porosity (for uptake of liquids) in pellets without loss of pellet durability; bearing in mind the increased handling of pellets when they are vacuum-coated.

To use vacuum coating as a consistent method of post-pellet liquid application, we should be able to consistently produce high quality and porous pellets. Vacuum coating has been used successfully in aquaculture feed and pet foods where end-product quality and nutrient density (e.g. up to 30% added fat) is important to both the animals and those feeding these diets. To achieve this, in the case of aquaculture and pets, the diets are often extruded rather than pelleted; although pelleted feed is also vacuum-coated when required. Pellets and extruded products have different functional properties due to the application of different processing conditions during production. The extrusion technique uses high temperature, moisture and pressure to achieve high durability, expansion and low density (high porosity) to make it favourable for aqua

feeding. However, with pellets, the density is high with lower porosity, which is a challenge to infusing high amounts of liquid during vacuum coating.

The hypothesis and objectives of the study are listed below.

1.1 Hypothesis

Our hypothesis were;

- the particle size of the diet is dependent on interactions between ingredients, grinding and pelleting and that this can be managed to improve porosity (and improve utilization for post-pellet addition of liquid(s) and bioactives) without losses in pellet quality;
- vacuum coating will maximize liquid uptake and protection of bioactives, and
- vacuum coating will increase the shelf-life of feed and bioactives.

1.2 Objectives

The objectives of this project were;

- to review the opportunities and challenges associated with vacuum coating of pelleted feed for broilers;
- to evaluate the effect of processing (particle size) on physical properties of pellets and the ability of pellets to absorb oil during post-pellet liquid application, and
- to evaluate the use of vacuum coating for protection of heat-sensitive bioactives (enzyme) during pelleting and storage.

2.0 LITERATURE REVIEW

2.1 Description of vacuum coating

Vacuum coating of pelleted or extruded feed is based on a simple physical exchange of air inside the pores of feed material with a liquid (Figure 2.1). The first step is to transfer cooled and dried extrudates or pellets to the vacuum coater, sealing the mixing vessel and creating a vacuum. The pellets are then gently mixed as the desired liquid material is pumped into the milieu and then the vacuum is released. This initially removes air from the pellets, and release of the vacuum causes the liquids to more deeply penetrate the pellet. The volume of feed and liquid, mixing, and vacuum conditions can be varied, but these need to be balanced with maintenance of pellet quality and diet throughput.

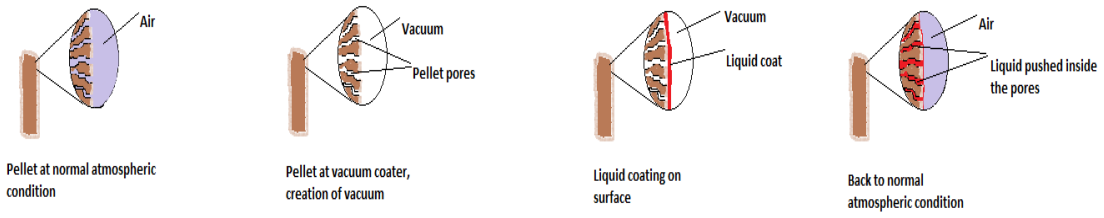

Figure 2.1 Principle of vacuum coating (Adapted from Perez, 2001)

Perez (2001) described the principles of vacuum coating for extruded feeds reporting that extrudates are dried to approximately 8% moisture and maintained at approximately 70°C when batched. Once the dried extrudates are placed in a vacuum coater, the air is then removed creating a vacuum in the pores of the extruded product. The liquid is sprayed over the extrudates and mixed to allow uniform coating. Once the liquid is coated on the surface of extrudates, the vacuum pressure is released allowing the liquid to be drawn into the extrudates. Lastly, the extrudates (~60°C) now full of liquid are sent to a cooler prior to packaging. Generally, the estimated duration to vacuum coat after batching is around two minutes; however, this will

depend on many factors. Based upon this principle, extruded aqua feeds and pet foods are supplemented with higher fat levels that improve performance and/or palatability while maintaining feed form. This principle applies for pellets too, however, it is felt the lower porosity of pellets as compared to extrudates creates a greater challenge to maintain pellet durability and uptake of high levels of liquids.

Vacuum coating enables higher inclusion of fat without compromising pellet durability (Borquez and Perez, 2007) and protection of heat-sensitive liquid ingredients (e.g., vitamins or enzymes) from heat and oxidation by air (Li et al., 2003). Perez (2001) has summarized the advantages of vacuum coating of extrudates and pellets as:

- Coating different products with different liquids;
- Higher level of liquid can be added after processing with minimal loss of feed structure;
- Liquids can be blended before addition to the products;
- Taste can be enhanced by adding flavors or masking detection of unpalatable additives inside the pellet.

Perez (2001) has also reviewed some features of vacuum coaters and how these impact product durability, capacity to facilitate good mixes in the short period that vacuum is applied, and the ease of cleanout and the importance of preventing cross contamination. There are not many references available about the use of vacuum coating to achieve these targets.

Although vacuum coaters are used extensively for extruded products in aquaculture and pet feed industries, there are also some references to vacuum coating of pelleted feed. Although there is some interest in extruding feed for other commercial livestock, the application is not extensive. Koeleman (2013) discussed a patented extruded pig feed processing that is being applied in Europe. With non-extruded products like broiler pellets, the application of vacuum

coating has certain challenges, such as its limited capability to absorb liquid due to lower porosity and the increase in cost of lower margin pelleted feeds. However, selection of ingredients and optimizing processing parameters can help to produce more porous and durable pellets for vacuum coating.

2.2 Potential uses of vacuum coating

2.2.1 Addition of fats

It is increasingly popular to maximize the utilization of co-products of oilseed or biofuel industries in broiler diets. Consequently, it is becoming more difficult to balance protein levels of diets with sufficient energy unless higher inclusion of oil or fat is allowed. To form a durable broiler feed pellets there are limits to fat inclusion (Thomas et al., 1998) through pre-pellet addition, generally from 3-5%. At higher levels of fat included in the mixer, the distribution of water may be altered and this can interfere with the solubility of proteins and/or gelatinization of starch that are associated with binding of pellets as well as nutrient availability. Fat or oil also acts as a lubricant and lowers the pressure that is achieved in the die and results in lower compaction of the feed ingredients into durable pellets. Salmon (1985) overcame some of the limitations of pellet quality with higher fat containing diets by using up to 2.5% sodium bentonite as a binder. However, as reviewed by Angulo et al. (1996), pellet binders are expensive and dilute the nutrients in the diet. Most formulations limit fat to less than five percent of the diet; therefore, when higher levels of fat are required, it is better achieved by using high fat ingredients (e.g. high oil corn or expelled whole soybean) due to the fat being "locked" in the ingredient matrix (Briggs et al., 1999). Nevertheless, it was found that total dietary fat exceeding 5.6% would deteriorate pellet quality regardless of the source. Thus, post-pellet

application of fats seems to be an effective means of maintaining pellet quality if higher inclusion of fats in the diet is imperative.

One of the simplest methods of inclusion of fat in pellets is spraying of the fat onto hot pellets immediately after pelleting (Strauch, 2002). However, post-pellet spray applications of fat are still limited with respect to the amount of fat that can penetrate the pellet without vacuum coating. The maximum amount of fat that can be sprayed should not exceed 2-3% at the pellet die, and 7 or 8% at truck load-out (Borquez and Perez, 2007). However, with vacuum coating, higher level of fats can be infused into the pellets without compromising its durability, handling and flow characteristics. In growing salmon diets, the use of vacuum coating enables incorporation of 34-40% added fat to extrudates and would not be feasible without vacuum coating (Bell and Koppe, 2010). The extrusion and expansion process of fish extrudates allow enough pore space to infuse such a high level of fat, and the extrusion process facilitates durable extrudates capable of withstanding the extra handling to vacuum coat.

Broiler diets are not extruded; therefore, the porosity and the degree of pellet durability are different. In a reference test [(International Research Association of Feed Technology, cited in Strauch (2002)] the level of fat inclusion was doubled in broiler pellets (die 3×30 mm, 3% steam) with vacuum coating and achieving up to 10% added fat. Borquez and Perez (2007) have successfully incorporated 5 to 7% more fat in pellets using vacuum coating without losses in pellet durability. Further, they have also mentioned various advantages of vacuum coating of fats to pellet feeds such as:

- Fat may be added to cold pellets, which are more durable than warm pellets;
- Up to 12% additional fat can be added to pelleted feed;
- Reduced leakage of added fat from the finished product;

- Cleaning and decontamination of vacuum coater equipment can be performed by a simple "washing step";
- The pellet quality (durability) can be enhanced due to improved elasticity of the pellet by higher fat or oil inclusion;
- Other bioactive liquids can be added along with fat; and
- With vacuum coating of fats, the diet no longer needs to include extra fat, which may improve pellet quality and throughput.

Therefore, vacuum coating can be an alternative method of increasing the energy of broiler pellets by post-pellet addition of fat without deteriorating the quality of pellets.

2.2.2 Protection of bioactives

In order to achieve optimum pellet quality, it is often necessary to increase the level of heat during conditioning (Spring et al., 1996; Cutlip et al., 2008; Abdollahi et al., 2010a) and thereby changing the functional properties of proteins and starch. However, this can be detrimental to bioactivity of heat-sensitive bioactives (e.g. enzymes, probiotics) or nutrient availability (e.g. vitamins, amino acids). High heat is also used to "hygenize" feed (Engelen and van der Poel, 1999) and not necessarily to improve pellet durability or nutrient availability. Added heat may also increases solubility of non-starch polysaccharides (i.e., increase digesta viscosity; Gracia et al., 2003) that reduces nutrient absorption and may increase wet litter and/or disease exposure. Consequently, the industry uses an excess of bioactives (e.g. NSPase, non-starch polysaccharidases) for reducing digesta viscosity associated with wet litter) to compensate for any loss during processing.

In the traditional post-pellet liquid coating system, most of the liquid ingredients are sprayed onto the surface of the pellets. During subsequent handling, any particles that break off, not only

contribute to the level of fines, but also concentrates the sprayed material in the fines. When these fines are recirculated for pelleting, the added bioactives may be destroyed by a second exposure to heat. Engelen and van der Poel (1999) reported that the absorption of enzymes by the pellet is very low and remains on the outer surface of the pellet. Due to these reasons, post-pellet liquid infusion system (i.e., vacuum coating) can facilitate application of heat-sensitive ingredients and secure better incorporation into the pellet (Li et al., 2003).

Vacuum coating may also improve protected delivery of bioactives to target gut absorption sites. The digestive tract of broiler consists of a diverse environment in terms of pH with ranges of 2.0 in the gizzard to 6.0 - 7.0 in the small intestine. β-glucanase (a NSPase, extensively used in barley-based diets) has lower activity in the presence of acids and digestive enzymes (Almirall and Esteve-Garcia, 1995). According to Svihus (2010), exogenous enzymes in feed are highly susceptible to hydrolysis while passing through the low pH of the proventriculus and gizzard before reaching the target site (small intestine). Svihus (2010) also noted that there has been limited success in adapting enzymes or coating of enzymes to protect them.

Similarly, short-chain fatty acids like butyric, propionic and formic acid are added to diets to reduce gut pathogens (Van Immerseel et al., 2005; Fernández-Rubio et al., 2009), improve immunity (Guilloteau et al., 2010), stimulate epithelial development (Panda et al., 2009) and to improve carcass quality in broilers (Leeson et al., 2005; Panda et al., 2009). However, these short-chain fatty acids are quickly absorbed in the upper gut (Thompson and Hinton, 1997) and this may be reduced with vacuum coating, thus facilitating intact delivery to the lower gut. Coating of short-chain fatty acids with protective layers has resulted in increased effectiveness (Van Immerseel et al., 2004, 2005; Boyen et al., 2008; Smulikowska et al., 2009). Vacuum coating may be a useful alternative tool to protect these bioactives from the harsh environment of

the digestive tract and to prevent absorption in the proximal gut. There needs to be more work done on determining if vacuum-coated bioactives in pellets would allow some protection from destruction in the gizzard through grinding in the presence of pepsin and acid.

2.2.3 Increase storage time

The shelf-life of food and feed is an important factor that impacts palatability or intake, loss of bioavailability and production of harmful metabolites, such as rancidity. There are few guidelines on shelf-life expectancy, except for food and mixed cereal products that are expected to have, in many instances, a shelf-life of more than 12 to 36 months. Vegetable oils commonly used in poultry diets are rich in polyunsaturated fatty acids that are more prone to lipid oxidation. Oxidation products decrease the nutrient composition of diets by reacting with proteins, lipids and fat-soluble vitamins (Tavárez et al., 2011). Further, oxidation products produce an unpleasant flavor in feed, contributing to lower voluntary feed intake. The negative effects of oxidised fat include decreased weight gain, feed efficiency, hatchability, meat quality and health (Cabel et al., 1988; Lin et al., 1989; Anjum et al., 2004; Upton et al., 2009). The risk of oxidation of fats occurs when fats or feeds containing high levels of fat are stored for a prolonged time. This can be reduced by protecting the fat (e.g. adding antioxidants) or by minimizing fluctuations in temperature and/or exposure to sunlight and/or air.

The activity of vitamins is also reduced in pelleted feed during storage (Marchetti et al., 1999). This work demonstrated that some crystalline vitamin activity was significantly reduced as storage time increased. Ascorbic acid and menadione (i.e., vitamin K) were reduced significantly after 90 days of storage and further reduction was observed at 180 days. In the case of pyridoxine (i.e., Vitamin B_6,) a significant reduction was noted only after 180 days. However,

coating of vitamins with fats prevented significant reduction of activity of most of the vitamins for up to 180 days, except in the case of ascorbic acid.

High temperature and prolonged storage of feed can also denature enzymes (Mascarell and Ryan, 1997). These losses are often exacerbated with surface applications during liquid additions after pelleting (Engelen and van der Poel, 1999). Post-pellet surface applications of enzymes increase direct exposure to destructive environmental conditions during handling and storage. Again, this is potentially exacerbated by the loss of enzymes in fines produced during the process.

To reduce losses of activity during storage, products are protected by use of antioxidants to control oxidation of fats and by coating of vitamins and enzymes. Through vacuum coating, these liquid additives can be infused deep into the pellet pores, thereby increasing the protection from exposure to heat, light and air, which are major initiation factors of lipid oxidation and bioactive destruction during storage. Studies regarding the use of vacuum coating to prove this concept are rare. Vacuum impregnation technology has been successfully used to produce Vitamin E and mineral fortified fresh cut apples to promote consumer acceptability even after seven days storage (Park et al., 2005). Therefore, there are certain opportunities of vacuum coating to be applied in pellet feed to increase its storage time.

2.2.4 Protected delivery of oral vaccines and probiotics

Vaccination is a means of reducing post-secondary infections or to control food borne pathogens (e.g., *Salmonella*) that otherwise would require antibiotics to control. With increased restrictions on the use of in-feed antibiotics, this needs to be explored further. However, unless a vaccine can be administered without individual bird handling, it is unlikely to be of significant use for the billions of broilers produced each year. Therefore, successful in-feed vaccination

would increase the arsenal of options to minimize disease and reduce reliance on antimicrobials. Further, in-feed vaccines reduces the need of trained personnel to vaccinate and may reduce biosecurity risks associated with mobile vaccination crews.

The features of an oral vaccine are its ability to be recognized as an antigen and not promote gut tolerance. In order to achieve this, the antigen must be protected in the feed and from destruction during storage, ingestion and digestion. One of the major limitations of oral vaccination is the instability of the antigen in the gut environment (Russell-Jones, 2000), so large amount of antigens have to be applied to elicit mucosal immunity through oral delivery of vaccines (Aizpurua and Russell-Jones, 1988). There have been some efforts to encapsulate oral vaccines to improve delivery to desired sites of gastrointestinal tract, however, with only limited success (Bowersock et al., 1996; Lavelle, 2006). In poultry there has been success in using feed to successfully deliver Newcastle Disease Virus (Olabode et al., 2010) and coccidiosis (Parry et al., 1992; Williams, 2002) vaccines.

Vacuum coating may be an alternative and less expensive method of encapsulation of oral vaccines. The effectiveness of vacuum coating to protect the vaccines and to deliver them to the target area of birds' digestive tract is not known. In these instances, it may be only necessary to provide a specific feeding of the vacuum-coated product rather than continual feeding that could be maintained with normal pelleted diets. Furthermore, because of this one feed exposure, the ingredients in the protected diet could be modified so as to reduce the challenges of the gut (e.g., negate low pH and protein enzymes; Muir et al., 2008) without a significant loss in performance. For probiotics that require more frequent delivery, vacuum coating may provide all the benefits described for vaccines and facilitate an economic method of delivery than encapsulation. One of

the challenges with adding encapsulated material in feed is their ability to withstand the challenges of pelleting.

2.2.5 Solve palatability issues

Apart from the unpleasant odor produced by the oxidation of polyunsaturated fatty acids, some feed additives impart a characteristically offensive odor in the feed (e.g., butyrate, a short chain organic acid with antimicrobial properties). This may affect worker safety and comfort and/or may be potentially corrosive to equipment, and in animals may result in feed refusal and losses in productivity. Thyme oil is another example; it is used for its antimicrobial effects and stimulation of metabolism. However, it has a disagreeable taste and its increased level in the diet causes decreased feed intake in young growing chickens (Cross et al., 2003). Similarly, caffeine and theophylline are feed additives that may be used to decrease the fat level in broiler meat, but have palatability issues (Jones et al., 1989). Vacuum coating of such feed additives into pellets could be a possible way to mask these effects on palatability and feed intake.

2.3 Challenges of vacuum coating of pellets

Despite the potential number of opportunities for vacuum-coated broiler pellet feeds, there are also a number of potential challenges. Vacuum coating technology has been used in the feed industry since the early 1990s. Even after more than two decades, its use is still limited primarily to aquaculture and pet feed. The challenges to apply this technology to obtain the previously mentioned benefits in broiler feed can be evaluated with respect to optimizing pellet functional properties and the higher costs associated with equipment and reductions in throughput. Unlike the use of extrusion in the production of fish extrudates, broiler feeds are pelleted. The extrusion process provides greater opportunities to manipulate the density of fish feeds and improve the floatation of the feed (Jovanovic et al., 2009). However, the high density

of pelleted feed is the major limitation for air-liquid exchange and reducing the uptake of liquid (Perez, 2001). Another more important challenge is the increase in cost of feed associated with vacuum coating. Pelleting of feed is itself an expensive process.

The use of vacuum coating of pelleted feeds needs investment of machinery, energy, time and labor. These factors contribute to an increase the price of feed and it is therefore imperative that vacuum coating provides cost benefits or a competitive advantage. Some examples where vacuum coating may justify the investment are reduced feed ingredient costs/choices, savings from the reduced need to over formulate vitamins or enzymes and improved performance, health and uniformity of birds. Competitive advantage may be realized with reduced use of antimicrobials and improved consumer acceptance.

2.4 Processing conditions and vacuum parameters affecting vacuum coating

The ideal pellets for vacuum coating should have sufficient porosity to provide adequate space for liquid addition and still maintain pellet durability. Selection of feed ingredients, manipulating particle size, changing processing conditions and altering the vacuum parameters can help to achieve this objective. Diet formulation is recognised to have a major impact on the physical pellet quality (durability and hardness). However, the impact of ingredients on porosity and the ability of pellet to uptake liquid by vacuum coating has not been reported. The effect of particle size on pellet durability has been variable, but may provide opportunities to maximize porosity to pelleted feed.

Various publications have recommended a variety of particle sizes to improve pellet durability (Engberg et al., 2002; Svihus et al., 2004a; Péron et al., 2005; Rodgers et al., 2012). This may be a function of differences in ingredients and/or conditioning temperature and moisture. Specifically for vacuum coating with vegetable oils, coarser particle size improved

outcomes associated with oil level inclusion and maintenance of pellet durability (Strauch, 2002). This can be attributed to the coarse particles providing more space and reducing the density of pellets. Furthermore, in the case of finer particle size, smaller particles occupy the large voids in between coarser material and consequently may decrease pellet porosity (Thomas and van der Poel, 1996).

Supplemental moisture decreases the bulk density of pellets (Moritz et al., 2002, 2003; Buchanan et al., 2010). The decrease in bulk density of pellets with higher moisture is attributed to an increase in pellet quality due to moisture addition, formation of more air space in intact pellets (after drying) and less fines (Moritz et al., 2003). In addition, supplemental moisture decreases friction in the pellet die, which results in less compacted and less dense pellets. Similarly, with the extrudates, porosity of the product increases as the moisture content (20, 30 or 40%) of extrudates increases (Umar et al., 2013). However, less compaction can make pellets less durable. The concept of effect of moisture on durability and porosity of pellets can improved by understanding the activity of moisture that will maximize pellet binding and durability and may improve porosity. When excess water is removed during cooling and drying, this would add to the porosity of the pellet.

Another important factor affecting the porosity of pellets is the pressure in the pellet die. With less pressure, there is higher porosity; however, this may significantly reduce pellet agglomeration and pellet durability. Li et al. (2003) found that smaller pellet size favored uniformity of liquid concentration in pellets after vacuum coating. This may be related to more uniform penetration of the liquid into the larger surface area of smaller pellets. Perez (2001) has also suggested to decrease the die compression ratio (Figure 2.1; Chao, 2010, that is associated with the ratio of length and diameter of the pellet die hole or opening) from 20 (length 60 mm

and diameter 3 mm) to 15 or 10 (length 45 or 30 mm). With a shorter pellet hole length, there is proportionally less resistance on the material being forced through the pellet die. In this case, lower die compression of the pellet may increase porosity and enable higher fat incorporation.

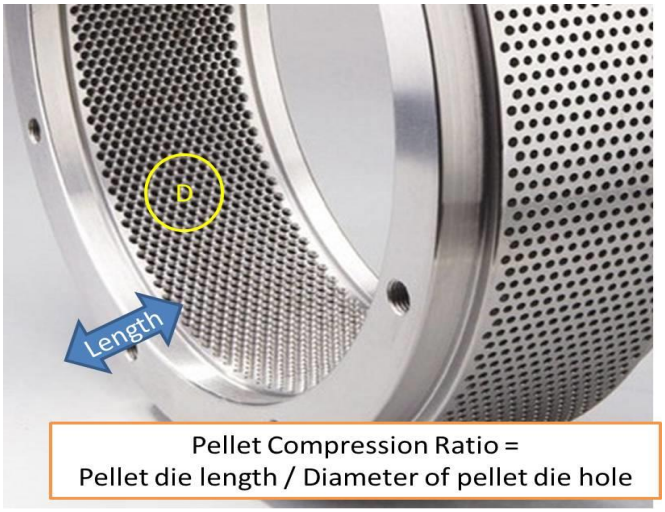

Figure 2.1 Illustration of pellet compression ratio (Chao, 2010)

According to Perez (2001), if there is no need for fat addition before or during pelleting, the compression ratio can be reduced to get less compaction and ultimately less dense pellets. There are also effects of the design and settings between vacuum coaters that need to be understood to more consistently achieve the desired level of liquid incorporation and to maintain uniformity of the product. Li et al. (2003) examined the effects of spray pressures (0.2, 0.4 and 0.6 MPa) and vacuum pressure (0.02, 0.03, 0.04 and 0.10 MPa) on mixing uniformity of liquids added to pellets. Similarly, the effects of liquid dosing (1, 2, 3, and 4% liquid by weight in feed), pressure release time (90, 180, and 270 sec) and vacuum pressures (0.01, 0.02, and 0.03 MPa) on concentration of liquid incorporation were examined. They found an improved uniformity of spray addition (i.e., lower CV; coefficient of variation) when spray was applied at 0.4 MPa. Vacuum pressure had no effect on the mixing uniformity. Improved uniformity of liquid applications was found when using shorter vacuum release time, higher liquid dosing and smaller

pellet size. Borquez and Perez (2007) found that the fat level in the pellet could be increased up to 12% by using the vacuum pressure of 500 mb (0.05 MPa) during vacuum coating. There are not enough studies on the effects of these variables on the efficiency of vacuum coating, thus studies targeted to understand the contribution of these processing steps is strongly recommended. Further, these studies also need to take into account the effect of variability in the ingredients used and how they are processed into pellets.

2.5 Assessment of pellets for vacuum coating

Highly durable pellets with adequate air space are needed to achieve high quality vacuum-coated pellets. There are several methods to assess the durability of pellets, which are regularly used in feed mills. Tumbling can (ASABE, 2012), Holmen pellet tester (Thomas and van der Poel, 1996), Ligno pellet test and Doris test (Aas et al., 2011) are some of the popular methods to determine durability of pellets. Another important physical property of pellet is its hardness that is commonly determined by using Kahl's pellet durability technology (Abdollahi et al., 2013). Durability reflects the production of fines from pellets during handling using equipment that provides repeatable assessment. Pellet hardness is the measurement of force the required to break the pellets. There is no universal acceptance of the procedures to measure pellet durability and hardness. Near Infrared Reflectance (NIR) spectroscopy has also been used to produce rigorous calibrations for predicting pellet quality (H. Eubank, Bruker Ltd., Atlanta, GA, personal communication, 2013). However, both hardness and durability are important physical attributes required so that pellets are able to withstand the extra handling and retain the liquid infused during vacuum coating.

There is no standard method to determine the porosity of pelleted feed. Porosity is generally defined as the fraction of bulk density of pellets that consists of air space. Therefore, if V is the

bulk volume of pellets and *Vs* is the volume occupied by solid matter; then, *V-Vs* is the total pore volume. Now porosity can be calculated as the ratio of *V-Vs* and *V*. There are several methods used to determine the porosity of rocks in geological sciences, tablets in pharmaceuticals and other porous bulk matter. Some of the methods are discussed here with the view of using these methods to calculate porosity of the pellets.

The mercury intrusion method measures displacement of air in a porous object by mercury (Giesche, 2006) due to the propensity for mercury (that is a non-wetting liquid with high contact angle; Rutledge et al., 2009) to penetrate pores under applied pressure. Similarly, gas pycnometry (i.e., specific gravity) is also a popular method to determine the porosity and density of grain kernels (Chang, 1988), fibres (Mwaikambo and Ansell, 2001) and other porous objects like rocks (Boving and Grathwohl, 2001). The gas pycnometer method is based on the ideal gas law (i.e., Boyle's gas law) that states, under isothermal conditions, the pressure and volume of a gas in a container remain constant. Therefore, differences in a given weight of material would estimate porosity. Gas pycnometry is commonly used to determine the porosity of rocks and the results are often compared with a water soaking method; it can also be applied to feed pellets and correlated with its fat absorption capacity (H. Christopher University of Saskatchewan, Saskatoon, SK, personal communication, 2013).

In the context of vacuum coating, the liquid absorption capacity of the pellet and ability to retain the liquid are more important than the actual determination of pore volume. However, the absorption capacity of pelleted feed is directly related to the pore space. Larger pore space in the pellets may result in leakage of liquids. Furthermore, excessive porosity, as discussed earlier, may reduce pellet durability. Sorensen et al. (2010) measured the effect of starch source and various processing conditions on expansion ratio, durability, fat absorption capacity and fat

leakage in extruded fish diets. To calculate fat absorption capacity, uncoated extrudates (600 g) were added to a lab-bench coater and the pressure lowered to 0.8 bar (0.08MPa). Following this, an excess of preheated (75°C) fish oil (400 mL) was sprayed into the coater during constant mixing, followed by release of pressure. Surplus oil was removed by gently pressing the vacuum-coated extrudates between 4-5 layers of oil absorptive lining. Then absorption capacity was calculated as weight (increase of pellet weight/initial weight) × 100 %. This method can also be used to estimate the pore volume. If the density of the liquid is known, then the weight increase of extrudates (weight of liquid absorbed and retained by the extrudate) when divided by density of liquid, pore space can be estimated. Sørensen et al. (2010) also determined the fat leakage in the same diet. Blotting paper was put into a plastic box and box was weighed. Fat coated extrudates (100 g) were placed in the above box (24 h at 40°C) and the amount of fat absorbed by the paper was used to determine oil leakage. Similarly, Øverland et al. (2007) used more material and a longer holding time to measure fat leakage from coated feed. The measurement of fat absorption and leakage seems to be a practical method to assess the effectiveness of vacuum coating of pelleted feeds.

Microscopic image analysis of pellets to evaluate porosity can be equally useful. There are number of microscopic techniques used to study the pore geometry of bulk matter, especially in petrology and medicine. Standard petrographic methods to analyze porosity of rocks use fluorescence microscopy (Yanguas and Dravis, 1985; Ruzyla and Jezek, 1987; Soeder, 1990). Generally, fluorescence dye is impregnated into the pores and manual and/or computer generated optical images provide an estimate of pore structure. These processes are relatively destructive and use of this technique may not be suitable for fragile pellets. Digital, multidimensional, high-

resolution image analysis offers precise and a more accurate estimate of the pore geometry (Mauko et al., 2009; Bagherzadeh and Latifi, 2013). However, these are expensive tools.

Near infrared reflectance (NIR) technology needs to be evaluated for its ability to estimate porosity and fat absorption. It has been successfully applied to estimate particle size and distribution in pharmaceuticals products (Ciurczak et al., 1986). It may also be a simple means of using NIR to measure the amount of liquid absorbed by the pellet with vacuum coating and thus estimating pore volume. Furthermore, NIR could be useful in assessing variability in ingredients and better match these with optimum process requirements to produce a more consistent pellet with the desired porosity and durability to better withstand vacuum coating.

2.6 Storage and shelf-life assessment of vacuum-coated products

It is important to assess the ability of vacuum coating to protect the added fats and liquid bioactives. One means of doing this would be the assessment of rate of oxidation or rancidity of fat applied with vacuum coating. The stability of fats is highly dependent on the storage conditions and type of fat added. There are a number of techniques available to test the oxidation of fat (Gray, 1978; Gordon, 2004). Similarly, the deterioration of enzymes and vitamins can be assessed by measuring the activity and/or concentration lost during storage. Shelf-life in some feeds may be in excess of six months, and would be best evaluated using a high holding temperature (e.g. >37°C) and with the expected exposure to air and light used during storage.

Assessment of water activity (a_w) is considered to be a useful tool for testing the physical, chemical and microbial stability of food and feed products. Rate of lipid oxidation is higher at either very low or high a_w as compared to the intermediate levels of water activity (Nelson and Labuza, 1992). Similarly, high a_w corresponds to a greater chance for bacteria and mould

growth. One of the important factors that determine the stability of vacuum-coated feed is the retention of infused liquid inside the pellet pores. The leakage of fats and other bioactives on the surface further accelerates the oxidation and loss of the bioactivity of nutrients. Thus, the assessment of feed stability and shelf-life would provide more information on the benefits of post-pellet liquid application with vacuum coating.

2.7 Summary

The use of vacuum coating in the feed industry is still largely applied only to high margin products such as extruded aquaculture and pet feed. However, with further research new uses for vacuum coating may become available, practical and economical for broiler pellets. Opportunities for vacuum coating pellets would be the ability to maintain pellet durability whilst markedly increasing the inclusion of fats, and reducing the loss of expensive bioactives and nutrients. However, to achieve this one has to be able to consistently produce pellets with both higher porosity and durability, and balance this with a better understanding of how to apply vacuum coating technology.

There are also opportunities that may exist for using vacuum technology for other processes, such as increasing the capacity to dry feeds or feed ingredients with less heat and thereby reduce the destruction of nutrients or bioactives. However, as with all other processes used in the feed industry, the value added must be balanced with the cost of using this technology.

3.0 EFFECT OF PARTICLE SIZE OF WHEAT, BARLEY AND CORN ON PELLET PHYSICAL PARAMETERS AND AMOUNT OF OIL ABSORPTION DURING POST-PELLET OIL APPLICATION WITH AND WITHOUT VACUUM COATING

3.1 Abstract

Vacuum coating (VC) is not usually associated with pelleted broiler feed. However, if vacuum coating can economically improve broiler performance, then pellet porosity and liquid supplementation will become increasingly important. An experiment was conducted to investigate the effect of particle size on post-pellet oil absorption, porosity, pellet durability index (PDI) and bulk density. Three different grains, wheat, barley and corn were either unground or ground with a hammer mill using two different screens to produce three particle sizes (whole, 3.2 and 6.4 mm hole size). The three particle sizes for each of the three grains were pelleted using a 4.7 mm die to obtain whole grain (WP), coarse (CP), and fine (FP) grind pellets. Following cooling, porosity, bulk density and PDI were measured before coating of pellets with an excess of canola oil (15% by weight) without (VC-) and with (VC+; 0.3 bar) vacuum coating. A complete random design (CRD) was used to analyse the effects of three particle sizes on PDI, bulk density and porosity separately for each grain (due to high variation in particle size between sources of grain). For oil absorption (OA), each grain type was analyzed separately using a CRD based 3×2 factorial design (three particle sizes; whole, coarse and fine; and two coating methods; VC- or VC+). With coarse grinding, the mean particle size was 1896, 1290 and 1057 µm, respectively for barley, wheat and corn; with fine grinding, the particle size was 1153, 767 and 732 µm, respectively. The PDI of WP for all grains was significantly ($P<0.01$) higher than CP and FP. There was no differences in the PDI of CP and FP in wheat. However, in barley, PDI of CP > FP ($P<0.01$) and in corn, FP>CP ($P<0.01$). For wheat, bulk

density of FP >WP= CP (P=0.05). For barley and corn, bulk densities of WP and FP were higher than for CP. Porosity of CP of wheat and corn was significantly (P<0.01) higher than WP and FP. However, for barley, there was no difference in porosity of CP and FP but both were significantly higher than for WP. For wheat, OA of CP was highest (P<0.01), but no significant difference was found between FP and WP. However, for barley, higher OA was found for FP followed by CP and WP. In corn, OA of CP was higher than for FP and WP. Vacuum coating (VC+) improved (P<0.01) OA of all pellets compared to VC-. Porosity varied among the ingredients and was positively correlated with OA and negatively correlated to PDI. Porosity was negatively correlated with bulk density. In conclusion, the grain type and particle size affected pellet physical parameters and oil absorption with vacuum coating increasing oil uptake.

3.2 Introduction

There are increasing amounts of low energy-high protein ingredients (e.g., co-products of oilseed or biofuel production) being used in broiler diets and as a consequence, more oil is needed to meet energy requirements. There are limits (~<5%) of oil that can be added in the mixer before pelleting (Briggs et al., 1999) or pellet durability index (PDI) will be reduced due to reduced friction in the die. Furthermore, fat in the mixer can limit starch gelatinization by altering water distribution in starch (Tester and Morrison, 1990; Zimonja et al., 2007). In these cases, higher oil inclusion is typically achieved by post-pellet application (Catala-Gregori et al., 2009).

There are several methods of post-pellet liquid application (PPLA) including spraying at the pellet die, mixing and coating of oils in a separate post-pellet mixer, and spraying at load out. The PPLA with fat is typically limited to inclusion of less than 8% of oil (Borquez and Perez,

2007). Vacuum coating is being used as a PPLA in aquaculture feeds and 30-40% of oil can be achieved when feed is extruded and highly porous (Bell and Koppe, 2010).

To vacuum coat, pellets or extrudates are batched into a vacuum coater mixer which is then sealed. A vacuum is created and the desired liquid(s) is added and mixed. Then the pressure is released and the liquid is efficiently drawn into the pores as a replacement for removed air. This process can significantly increase oil incorporation into the feed with minimal loss of PDI. However, with higher density (i.e., less porous) pellets, liquid infusion by vacuum coating is limited.

The present study will determine how grain source and particle size can be manipulated to achieve durable pellets with higher porosity to facilitate increased PPLA of oil. Porosity provides space for liquid infusion and lowers density. However, low density and highly porous pellets may have low PDI. An understanding of the effects of ingredients and processing conditions could provide better discernment of these complex interactions.

3.3 Materials and Methods

3.3.1 Grinding and particle size measurement

For each of three grains (wheat, corn and barley) there were three grind sizes (whole, 3.2 (fine) and 6.4 (coarse) mm hole size) compared after grinding using a hammer mill (Glen Mills, GH1 2688, Maywood, NJ, USA). The ASABE (2012) sieve sorting method was used to determine mean particle size (d_{gw}; geometric mean diameter). The test sieves (USA 203-mm, W.S. Tyler, Mentor OH, USA) no. 6 (3.35 mm; aperture size), 8 (2.36 mm), 10 (2.00 mm), 12 (1.7 mm), 16 (1.18 mm), 20 (850 µm), 30 (600 µm), 40 (425 µm), 60 (250 µm), 80 (180 µm) and Rotary Lab Sifter (Hoskin Scientific Ltd., G-213-HM-300, Burnaby, BC, Canada) were used to determine particle size for each sample in triplicate. A 100 g of sample was placed in top

sieve and shaken for 10 min in sieve shaker. Then, weight of material on all sieves was recorded. Mean particle size (geometric mean diameter) of the sample was calculated by using the formulas described in ASABE (2012).

3.3.2 Pelleting

For each grain type, the three particle sizes were pelleted using a 4.7 mm die (CPM P/N 3-3093-15, Crawfordsville, IN, USA). For fine and coarse corn, steam (1.4 kg/hr rate; 35 psi) was used to get sufficient pellet sample for analysis. The conditioning temperature and retention time were 75-80°C and 30 sec, respectively, for all grain types.

3.3.3 Bulk density and pellet durability index

The bulk density (kg/m^3) was determined (ASABE, 2012) after correction for dry matter. The Holmen Pellet Durability Tester (Borregaard, UK Ltd. LT 218) was used to measure PDI (% intact pellets). Briefly, 100g of screened (2 mm) pellets were pneumatically agitated for 30 sec and the pellets retained in the screen were weighed to obtain the durability reading. As coarse ground or whole kernels (e.g., >2mm particle size) were used to make pellets, a modified PDI was achieved by sieving the remaining material through a 4 mm screen to differentiate pellets from large particles.

3.3.4 Post-pellet liquid application (PPLA)

The absorption of oil during vacuum coating was determined as described by Sorensen et al. (2011) in fish extrudates. Cooled pellets (500 g) were placed in a prototype vacuum coater (Figure 3.1 and 3.2) and 0.3 bar (0.03MPa) pressure (~30 sec) was achieved inside coater. Then preheated (70°C) canola oil (75 g; 150g/kg feed) was injected while pellets were gently and continuously stirred to achieve even coating of pellets (~20 sec). Vacuum pressure was released (~15 sec) to complete the vacuum coating process. The coated pellets were then placed on

absorbent papers (4-5 layers) to remove excess oil and the oil absorption capacity determined (Equation 1). Oil absorption capacity without vacuum coating was calculated following the same procedure, except without reducing the pressure.

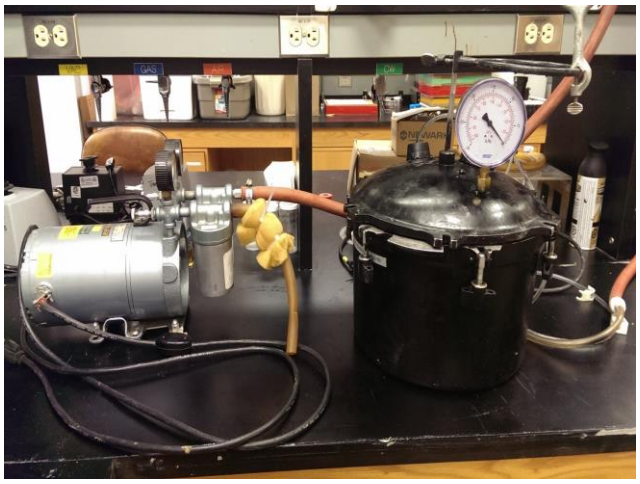

Figure 3.1 Experimental vacuum coating system

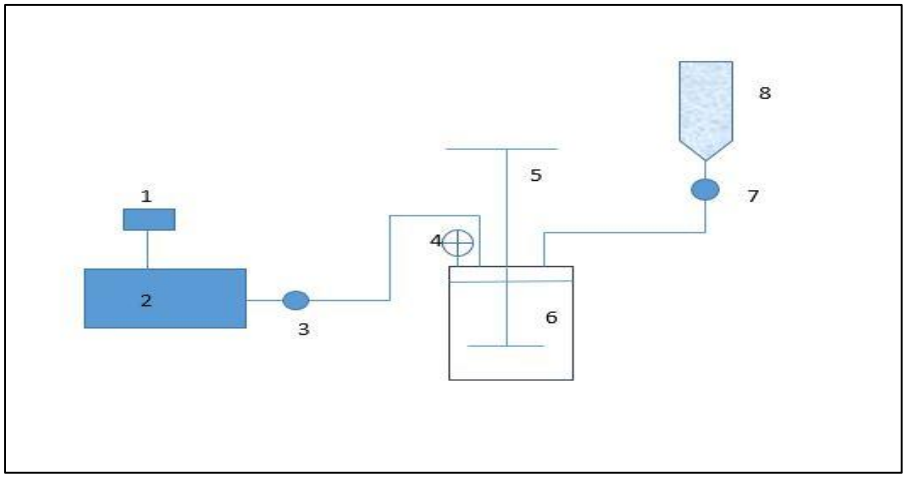

Figure 3.2 Experimental vacuum coating system. 1. Pressure control. 2. Vacuum pump. 3, 7. Valve. 4. Pressure gauge. 5. Mixture. 6. Vacuum chamber. 8. Liquid tank

Equation 1 $$\text{Oil absorption} = \frac{\text{Final weight} - \text{initial weight}}{\text{initial weight}} \times 100\%$$

3.3.5 Porosity measurement

The porosity of the pellets was calculated by measuring the bulk and unit volume of the pellets using a gas porosimeter (Coretest system, TPI-219, Morgan Hill, CA, USA). The bulk volume of 6-7 pellets was measured using digital caliper (Titan 23175, Renton, WA, USA). Unit volume of same pellets was measured using a gas (nitrogen) pycnometer. Then, porosity of pellets was calculated (Equation 2).

a b

Figure 3.3 Porosity measurement. a. Gas porosimeter to measure unit volume. b. Digital caliper to measure pellet bulk volume

Equation 2 $$\text{Porosity} = \frac{\text{bulk volume} - \text{unit volume}}{\text{bulk volume}} \times 100\ \%$$

3.3.6 Statistical analysis

Proc MIXED model of SAS version 9.3 (SAS Institute Inc, 1996) was used for data analysis. Particle size of the different grains are analysed for coarse (6.4 mm screen) and fine (3.2 mm screen) to evaluate the effect of screen size on particle size. For each type of grain pellet, bulk density, PDI and porosity, analysis were based on six replicates for each of the three particle sizes. The data for each grain was analyzed separately due to the difference in particle sizes among sources of grain. For oil absorption capacity (n=4), analysis was carried out using a 3×2 factorial design based on three particle sizes and two coating methods; again, analysis for each

grain source was conducted separately for each grain source. Means separation was conducted using Tukey's test. Correlations (Pearson) among the measurements were analysed by combining the values for all grains and particle sizes and Proc CORR method of SAS 9.3 was used. The study assumed that significance is achieved when $P \leq 0.05$ and trends would be assumed if $P > 0.05$ and $P < 0.10$.

3.4 Results and Discussion

3.4.1 Particle size

Due to the differences in particle size when grinding the different grains, data were analyzed separately for parameters associated with physical characteristics and oil absorption. For coarse grinding, particle size was highest for barley (1896 µm) followed by wheat (1290 µm) and corn (1057 µm). For fine grinding, there was no significant difference in the particle size of wheat and corn, but both had smaller particle sizes than barley (Table 3.1). The differences between particle sizes for each grain source were calculated; there was a 40.5% difference when coarse vs fine screens were used for wheat, 38.9% for barley and 30.7% for corn.

Table 3.1 Mean particle size of wheat, barley and corn after hammer milling using two screen sizes (3.2mm and 6.4 mm) with hammer mill (n=6)

	Grain particle size (µm)				
Hammer mill screen size	Wheat	Barley	Corn	*p-value*	*SEM*[1]
Coarse (6.4 mm)	1290[b]	1896[a]	1057[c]	*<0.01*	*21.9*
Fine (3.2 mm)	767[b]	1153[a]	732[b]	*<0.01*	*21.5*
Change (%)	40.5	38.9	30.7		

[a-c] Means within rows with no common superscripts are significantly different ($P \leq 0.05$)
[1]standard error of mean

There are a number of factors that determine the milling properties of grain. Grain hardness and vitreousness have a considerable effect on wheat milling behaviour and flour particle size

(Haddad et al., 1999). Dziki and Laskowski (2004) demonstrated that the size of wheat kernels (large and medium-sized kernel yielded a smaller particle size distribution) also influences the grinding properties of wheat. Chemical composition can have an impact on the hardness characteristics. Hardness is defined as the resistance of the grain to crushing (Lesage et al., 2012) which impacts the energy required for grinding and distribution of particle size of ground grain (Konopka et al., 2005). Hardness is also affected by kernel density, bulk density and its susceptibility to breaking prior to grinding because of post-harvest processing (Williams et al., 2009). For wheat, the puroindoline proteins play a crucial role in the hardness of the grain (Lesage et al., 2012). In addition, the kernel density of wheat affects its hardness and grinding characteristics (Konopka et al., 2005). For corn, the ratio between glassy and floury endosperm influences the degree of hardness (Williams et al., 2009). The protein in corn can influence the starch granule packing density within the kernel (Williams et al., 2009). This can also happen in wheat and barley, but due to different classes of protein being involved there can be differences in the degree of packing and hardness. There is limited work investigating differences in particle size between grain sources and the nature of grinding despite the fact that screen selection is important to obtain a specific particle size using different sources of grain. Barley bran shatters to a greater extent when rolled than wheat barn (Jadhav et al., 1998), and ground barley has distinguishable specks of bran (Baik and Ullrich,, 2008) which explains the higher mean particle size. Differences in the particle size of finely ground wheat and corn were non-significant (Table 2), which could explain the use of fine screen sizes in industry to reduce the effect of grain source on final particle size.

3.4.2 Pellet durability index (PDI)

There was a significant effect of particle size on the pellet durability index (PDI) for all of the grains tested (Table 3.2). There are many studies that report the impact of particle size on PDI, however the results are inconsistent (Sivhus et al., 2004; Peron et al., 2005, Amerah et al., 2007). The inconsistent effects may be a function of source of grain, diet formulations and the conditions used for pelleting. Results may also depend on the method used to measure durability.

The PDI of whole grain pellets was higher than for coarse and fine grind pellets. The higher PDI (Table 3.2) in whole grain (wheat and barley) was mainly due to the larger particles retained in the screen of durability tester. This was corrected by using a larger screen in the determination of the modified PDI (MPDI; Table 3.2). These PDI values might not reflect the true durability when the fines (produced from pellets during testing) are too large to pass through the durability tester screen. The MPDI was determined for all pellets by screening the pellets and large particles remaining in the test chamber. This resulted in a significant reduction in PDI (MPDI) for whole grain pellets, with the exception of whole corn pellets. However, in this study it was necessary to use steam when pelleting coarse and fine corn pellets. This can explain the difference in the durability associated with corn as compared to wheat and barley. It has been already reported that steam addition improves pellet quality (Gilpin et al., 2002; Abdollahi et al., 2012). In contrast, the whole pelleted corn was produced without steam and had the highest PDI (95.1%). This can be explained as larger corn kernels provided more resistance at the pellet die and this increased the pressure required for producing a more durable pellet. The energy usage for pelleting of each treatment was not available in present study, but should be noted in future work.

Table 3.2 Effect of particle size on physical parameters (PDI, pellet durability index; MPDI, modified PDI; bulk density; and porosity) of wheat, barley and corn pellets (n=6)

		Grains		
Measurement	Particle size	Wheat	Barley	Corn
PDI (%)	Whole	32.1[a]	59.0[a]	95.1[a]
	Coarse	14.3[b]	28.9[b]	77.5[c]
	Fine	11.6[b]	14.6[c]	86.1[b]
P-value		*<0.01*	*<0.01*	*<0.01*
SEM [1]		*0.85*	*0.54*	*0.66*
MPDI (%)	Whole	11.6[ab]	14.2[a]	94.4[a]
	Coarse	12.6[a]	4.0[b]	75.8[c]
	Fine	10.5[b]	12.5[a]	84.7[b]
P-value		*<0.01*	*0.04*	*<0.01*
SEM		*0.37*	*0.65*	*0.77*
Bulk density (kg/m^3)	Whole	802.9[b]	791.5[a]	872.2[a]
	Coarse	812.0 [ab]	760.6 [b]	816.3[b]
	Fine	824.4 [a]	775.4[ab]	848.8[a]
P-value		*0.05*	*<0.01*	*<0.01*
SEM		*5.68*	*4.60*	*8.84*
Porosity (%)	Whole	12.7[c]	13.2[b]	6.6[c]
	Coarse	15.8[a]	17.2[a]	11.4[a]
	Fine	13.9[b]	17.1[a]	8.9[b]
P-value		*<0.01*	*<0.01*	*<0.01*
SEM		*0.31*	*0.73*	*0.42*

[a-c] Means within columns and response variable with no common superscripts are significantly different (P≤ 0.05)
[1] Standard error of mean

3.4.3 Bulk density

The average bulk densities of different pellets are presented in Table 3.2. The bulk density of pellets is an important physical characteristic in regards to the economics of transportation and storage. However, more dense pellets may affect the degree of liquid infusion possible during PPLA. In wheat, the fine grind pellets had higher bulk density than whole grain pellets, but neither was different from coarse grind. For barley, the whole grain pellets had higher bulk density than coarse grind pellets but these values were not different from those of fine grind

pellet. The bulk densities of whole corn pellets and fine grind corn pellets were not different, but both were higher ($P<0.01$) than coarse grind pellets. The variable density measured in the pellets from different particle sizes could be due to variability in the distribution of particles in the respective pellets.

In barley and corn, fine grinding may increase the compaction in pellets, whereas coarse grinding may result in more air space between adjacent particles and reduce the bulk density. However, this was not the case for wheat pellets, which may be due to the impact of grain hardness as discussed above, resulting in a different pattern of particle size distribution or less compaction of particles during pelleting. There is limited information available about the effect of particle size on bulk density, but the density of pellets plays important role in liquid infusion during vacuum coating (Perez, 2001).

3.4.4 Porosity

The porosity of pellets is summarized in Table 3.2. The porosity of whole grain pellets was ($P<0.01$) lower than for coarse or fine grind pellets for all of the grain sources. Similarly, for wheat and corn, the porosity of coarse pellets was higher ($P<0.01$) than for fine pellets. However, for barley, the pellet porosity, as a result of coarse and fine grinding was not different. No studies were found that measured the effect of particle size on pellet porosity. The lower porosity in the whole grain pellets might be due to use of whole kernels as these large intact pieces of grain would have lower porosity than ground and pelleted sources. In the case of coarse pellets, as described above, large irregular particles may create larger pores between particles and explain the higher porosity, and vice versa for fine ground pellets. The reason for the inconsistent results with barley pellets is not obvious, although the hardness of the barley particles may be important. However, it may also relate to inaccuracy in measuring the pellet

dimension and thus the porosity values, especially when pellets are weak and do not have a smooth surface.

3.4.5 Oil absorption

For all three grain sources analysed, both the particle size and coating method had significant effects on the amount of oil absorbed (Table 3.3). For wheat, pellets from coarse grind had higher (P<0.01) oil absorption (9.2%) than fine (7.9%) and whole (7.5%) grind pellets. However, for barley, fine grind pellets had (P<0.01) higher (10.6%) oil absorption than whole and coarse grind pellets.

Table 3.3 Effect of particle size and coating methods on oil absorption of wheat, barley and corn pellets

			Oil absorption (%)		
Items	Level	*n*	Wheat	Barley	Corn
Total		24	8.3	9.47	5.5
Particle size					
	Whole	8	7.5^{b}	8.1^{c}	3.7^{c}
	Coarse	8	9.2^{a}	9.6^{b}	7.5^{a}
	Fine	8	7.9^{b}	10.6^{a}	5.2^{b}
SEM			0.16	0.09	0.24
Coating					
	VC- [1]	12	7.6^{b}	8.7^{b}	4.8^{b}
	VC+ [2]	12	8.8^{a}	10.3^{a}	6.2^{a}
SEM			0.13	0.07	0.20
P-values of main effects and interactions		*df*			
Particle size		2	<0.01	<0.01	<0.01
Coating		1	<0.01	<0.01	<0.01
Particle size*coating		2	0.79	0.09	0.69

[a-c] Means for particle size within columns and main effects with no common superscripts are significantly different (P≤ 0.05)
[1] Without vacuum application, [2] With vacuum application

There was a trend (P=0.09) for a significant interaction between particle size and coating methods for oil absorption of barley pellets (Figure 3.4). The two-way interaction indicates that there were significantly higher oil absorption with vacuum coating (VC+) of barley pellets when whole, coarse and fine grind comparisons were made. The trend for the interaction could be due to the differences in degree of oil absorption for VC- and VC+ with different grind sizes. In the

case of corn, like wheat, coarse particle pellets had higher oil absorption than the fine or whole pellets. Vacuum coating significantly ($P<0.01$) improved oil absorption for all grains.

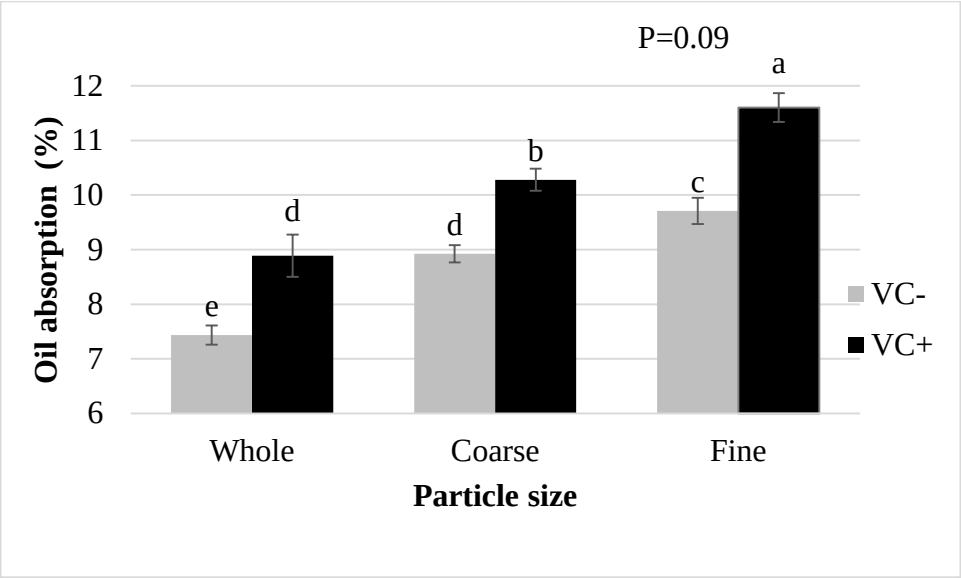

Figure 3.4 Interaction of coating methods and particle sizes on oil absorption of barley pellets, n=4 (VC-; without vacuum, VC+; with vacuum). Bars with different letters (a-e) are significantly different.

The significant impact of particle size on oil absorption may be due to variable particle size distribution and different particle assembly within the pellets. In the case of wheat and corn, coarse grind pellets absorbed more oil than pellets formed from whole and fine grind pellets. This can be attributed to coarse particles providing more air space between the adjacent large irregular particles. In a reference test (International Research Association of Feed Technology, as cited by Strauch, 2002), higher oil absorption was found when pellets produced from coarse mash were vacuum-coated. Furthermore, Strauch (2002) noted that pellets produced from coarsely structured mash had higher porosity. However, in the current study, the coarse ground barley pellets had lower oil absorption than the fine grind treatment. This may be related to the larger particle size (1896 µm) of coarse ground barley grain, facilitating looser binding of pellets

and post-coating oil leakage. Further, in barley, coarse grind pellets were characterized by the presence of large hull particles, which reduced the retention of oil.

In the present study, the relative oil absorption after vacuum coating was 16, 18 and 29% higher for wheat, barley and corn pellets, respectively, than without vacuum coating. However, the amount of oil absorbed in the pellets of present study was lower than other studies, but these were based on whole diets. Borquez and Perez (2007) successfully increased the oil level up to 12 % in pelleted feed. In a reference test (Strauch, 2002), 10% oil inclusion was achieved using vacuum coating. However, there were some differences in equipment and calculation of oil absorption. This study considered the loss of oil after coating, for example, and this may have differed with other studies. Further, oil absorption varies depending on ingredients, vacuum parameters (vacuum pressure, spray pressure, mixing time) and the capacity of pellets to hold the infused liquid.

3.4.6 Relationship of fat absorption capacity, porosity, PDI, modified PDI and bulk density

The Pearson correlation (r) coefficients for oil absorption, PDI, modified PDI, porosity and bulk density are shown in Table 3.4. There are positive correlations between porosity and oil absorption in all of the grain sources. Similarly, PDI is negatively correlated to porosity. Bulk density is negatively correlated to porosity. Higher porosity provides adequate space for liquid absorption during PPLA, and fat absorption is higher.

Table 3.4 Pearson correlation coefficient (r) of oil absorption, Pellet durability index, modified pellet durability index, porosity and bulk density (n=36), data combined for wheat, barley and corn.

	Porosity	Bulk density	Oil absorption	PDI
Bulk density	-0.78**	1.00		
Oil absorption	0.91**	-0.81**	1.00	
PDI	-0.82**	0.56**	-0.79**	1.00
MPDI	-0.81**	0.68**	-0.79**	0.91**

** P<0.01

In each of the pellets, the porosity value is higher than the oil absorption value (Table 3.2 and 3.3). This may be because, the oil applied did not have access to all of the voids available inside the pellets. Alternatively, excess pore space may have resulted in oil leakage. The negative correlation between pellet bulk density and oil absorption is explained by less dense pellets having higher porosity that accommodates more oil absorption during PPLA. Similarly, more porous and less dense pellets are less durable, absorb more oil, but are less desirable due to reduced PDI.

3.5 Conclusion

Vacuum coating technology was used successfully to inject oil inside pellet pores. This technology increased the amount of oil inclusion in comparison to coating without vacuum. Grain type had an effect on the physical parameters and oil absorption. Therefore, selection of ingredient can be an important consideration for the use of post-pellet vacuum coating. Particle size significantly affected the amount of oil inclusion with coarse particle size pellets absorbing more oil during coating. Similarly, particle size had a significant impact on bulk density, PDI and porosity of pellets. The use of gas pycnometer to measure porosity proved to be a useful measure of oil absorption as values were highly positively correlated. There is still more work to be done to improve the use of vacuum coating as a method of PPLA for pellets. In particular, comparisons of multiple ingredients pellets with varying porosity and durability need to be tested. It is also important to see the effect of different processing variables on porosity, durability and oil absorption.

4.0 EVALUATION OF POST-PELLET VACUUM COATING ON PROTECTION OF XYLANASE AND EFFECT ON BROILER PERFORMANCE WHEN FED WHEAT-RYE-BASED DIETS

4.1 Abstract

An experiment was conducted to evaluate the effect of vacuum coating (VC) on post-pellet application of xylanase and its impact on broiler performance when fed wheat-rye-based diets. Twelve diets were produced based on three enzyme addition methods [(EAM; without enzyme (E-), enzyme added pre-pelleting (PreE+) and post-pellet enzyme addition (PosE+)), two pellet conditioning temperatures (CT; 65 and 95°C) and two coating methods (CM; without (VC-) and with (VC+; 0.3 bar, 1 min mixing) vacuum application)]. Enzyme activity, diet extract viscosity and pellet durability index (PDI) of pellets were determined. The diets were fed to 360 Ross 308 male broilers in two phases (starter; 10-21d (5 birds×6 cages/diet) and grower/finisher; 21-35d (5 birds×3 cages/diet)) to determine performance and digestibility. A completely randomised block design based on 3 EAM x 2 CT x 2 CM was used. Enzyme activities of post-pellet enzyme coated diets were ($P=0.04$) improved by VC+. High CT significantly ($P<0.01$) improved PDI. There was a significant ($P=0.03$) 3-way interaction for 21d body weight (ABW), whereas at 35d only PreE+ and PosE+ improved ($P<0.01$) BW. There were no significant treatment effects on feed intake of starter; however, 95°C CT reduced ($P<0.05$) feed intake of grow/finish diets. Feed efficiency was improved ($P<0.05$) by EAM (PreE+ and PosE+) and 95°C CT. There was a 3-way interaction ($P=0.04$) on digesta viscosity (cP) at 21d (starter diets), whereas only EAM ($P<0.05$) was significant at 35d. Enzyme addition (PreE+ and PosE+) and high CT increased ($P<0.05$) apparent metabolisable energy (AME) of both starter and grower diets. Vacuum coating significantly ($P<0.05$) reduced the relative length of small intestine of broilers at 21d, but

not at 35d. In conclusion, VC may offer a means of delivery of heat-sensitive bioactives in broiler pellets; however, more studies are recommended.

4.2 Introduction

Broiler feed is generally pelleted and the use of high conditioning temperature (> 90°C) is preferred to maintain pellet quality (Cutlip et al. 2008), hygenize feed (Engelen and van der Poel, 1999), inactivate anti-nutritional factors and improve digestibility of the ingredients and performance. However, one of the challenges encountered with the use of high conditioning temperature in a cereal-based diet is a potential increase in digesta viscosity (Gracia et al., 2003; Cowieson et al., 2005) due to increased solubility of non-starch polysaccharides (NSP). Highly viscous gastrointestinal content reduces feed passage rate, diffusion of nutrients, favours microbial growth in the gut and consequently reduces performance (Choct and Annison, 1992; Inborr and Bedford, 1994). Bedford and Classen (1992) reported a negative correlation between digesta viscosity and growth performance. Wheat and barley have variable amounts of NSP depending on the cultivar and growing conditions (Scott et al., 1998). The use of exogenous xylanase enzyme in wheat- and/or barley-based diets in poultry has become a common practice to counter the problem of viscosity. However, with the use of a high processing temperature, the retention of activity of added enzyme can be reduced (Eeckhout et al., 1995). Post-pellet addition of enzyme can overcome the problem of inactivation (Silversides and Bedford, 1999) but homogeneity of enzyme application is difficult to achieve. Furthermore, most of the applied enzyme remains on the outer surface and absorption of enzyme into the pellet is low (Engelen and van der Poel, 1999). Consequently, the applied enzyme may be concentrated in the fines and bioactivity further diminished during re-pelleting. Another challenge is to protect the enzyme before its delivery to the target a specific gut location for delivery, as low pH in the

proventriculus and gizzard will hydrolyze certain enzymes (NSPase; Svihus, 2010) and reduce efficacy in the small intestine. In addition, storage of feed can result in losses of bioactivity due to exposure to external elements.

In these instances, use of vacuum coating of enzymes as a post-pellet application method can be useful. Vacuum coating would "bury" enzymes inside the pores of the pellet, thereby protecting the enzyme and reducing the degree to which enzymes were concentrated in the fines. There have been studies conducted on the use of vacuum coating to infuse higher level of fats in extruded products. However, there is little information available on its use for protected delivery of bioactives. This study is focused on the use of vacuum coating technology as an alternative method of post-pellet enzyme (xylanase) application, and its effect on the retention of enzyme activity after processing and the performance of broiler chickens.

4.3 Materials and Methods

All experimental protocols and procedures were approved by the Animal Care Committee (animal use protocol no. 19940248) at the University of Saskatchewan and care of the birds was in accordance with the recommendations of the Canadian Council of Animal Care (1993). Chicks were monitored a minimum of twice daily throughout the trial for comfort, availability of feed and water, and mortality. Unthrifty or unhealthy chicks were humanely killed by cervical dislocation.

4.3.1 Diets

From 0-10d, a commercial crumbled starter feed (Whole Earth Chick Starter, Federated Cooperative, Saskatoon, SK,) was provided. This was done as the focus of the present study was on pelleted feed, not crumbled feed required for young (<10d) chicks. There were 12 experimental diets fed as pellets using two diet phases, starter (10 to 21d) and grow/finish (21 to

35d). The composition and calculated nutrient analysis of the experimental diets are given in the Table 4.1.

Table 4.1 Composition and calculated analysis of basal diets (g/kg) as fed

Ingredient	Starter (0 to 21d)	Grow/Finish (21-35d)
Wheat	584.3	643.9
Soybean meal (48%)	200.0	140.0
Rye	103.2	113.6
Canola oil[a]	50.0	50.0
Fish meal	20.0	10.0
Limestone	12.0	12.0
Dicalcium phosphate	5.0	5.0
DL-Methionine	5.0	5.0
Lysine – HCl	4.0	4.0
Salt	2.5	2.5
Choline chloride	1.0	1.0
Broiler vitamin-mineral premix[b]	5.0	5.0
Celite – acid insoluble ash marker	8.0	8.0
Liquid xylanase (25,000 Xylanase units/g)[c]	128g/ton	128g/ton
Calculated Analysis		
Metabolisable energy (kcal/kg diet)	2990	3030
Crude protein	220.0	197.5
Fat / Ether extract	70.0	70.6
Lysine	14.5	12.1
Methionine	7.7	7.3
Calcium	7.5	6.4
Phosphorus - available	3.8	3.2

[a] 10g/kg (1%) canola oil was added in the mixture before pelleting. In diets with pre-pellet enzyme addition, enzyme was mixed with oil and added. Remaining 40 g/kg (4%) was added post pelleting with or with enzyme/with or with vacuum depending upon the treatments.

[b] Supplied per kg of diet: vitamin A, 11000 IU; vitamin D3, 2200 IU; vitamin E, 30 IU; thiamine, 1.5 mg; riboflavin, 6mg; niacin, 60 mg; vitamin B_6, 4.0 mg; vitamin K, 2.0 mg; vitamin B_{12}, 20.0 mcg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg/kg; biotin, 150 mcg; iron, 80 mg; copper. 10mg; manganese 80 mg; selenium 0.3 mg

[c] Diets were made either without or with liquid xylanase depending upon the treatments. It was added either pre-pelleting or post pelleting. Enzyme was obtained from Canadian-Biosystems, Calgary, Canada with the following technical specifications: Product name: liquid xylanase; Activity: 25,000Xyl units/g; source strain: *Trichoderma reeesi*; Ingredients *Trichoderma reesei* fermentation extract, water, glycerin, sodium chloride, potassium sorbate, sodium benzoate; Application details: Enzyme supplement for use in animal feeds. Liquid format for application post-pelleting

4.3.2 Feed Processing and vacuum coating

Experimental diets were manufactured at the Canadian Feed Research Centre, North Battleford, SK, Canada. The wheat and rye used in the diet were first batched and then ground through 4.57 mm screen in a hammer mill (G.J. VIS, Model: VISHM2014, Oak Bluff, MB, Canada). Then the ingredients were mixed for 1 min in a twin-shaft paddle mixer (UAS-Muyang, Model: SLHSJ, Abbotsford, B.C, Canada). There were three batches (500 kg each for both starter and grow/finish) of diets made and pelleted. The oil and enzyme additions were different among diet batches and are illustrated in Table 4.2, and explained in detail below.

Table 4.2 Description of experimental diets showing differences in enzyme addition, temperature and coating methods.

Diet No.	Treatment	Enzyme Addition Method (EAM)	Conditioning Temperature (CT) (°C)	Coating Method(CM)[1]
1	E-_65_VC-	No Enzyme(E-)	65	VC-
2	E-_65_VC+	No Enzyme(E-)	65	VC+
3	E-_95_VC-	No Enzyme(E-)	95	VC-
4	E-_95_VC+	No Enzyme(E-)	95	VC+
5	PreE+_65_VC-	Enzyme Pre-Pellet (PreE+)	65	VC-
6	PreE+_65_VC+	Enzyme Pre-Pellet (PreE+)	65	VC+
7	PreE+_95_VC-	Enzyme Pre-Pellet (PreE+)	95	VC-
8	PreE+_95_VC+	Enzyme Pre-Pellet (PreE+)	95	VC+
9	PosE+_65_VC-	Enzyme Post-Pellet (PosE+)	65	VC-
10	PosE+_65_VC+	Enzyme Post-Pellet (PosE+)	65	VC+
11	PosE+_95_VC-	Enzyme Post-Pellet (PosE+)	95	VC-
12	PosE+_95_VC+	Enzyme Post-Pellet (PosE+)	95	VC+

[1]VC-; without vacuum (atmospheric pressure in vacuum coater), VC+; with vacuum (0.3 bar (0.03 MPa) pressure in vacuum coater)

For the first series of four diets (Table 4.2; Diet No. 1 to 4) no enzyme was added either pre- or post-pelleting. The diets were conditioned (3 barrel conditioner, Model: MUTZ350-J, UAS-Muyang, Abbotsford, B.C, Canada) at 65 or 95°C before pelleting (UAS-Muyang, Model: MUZL310II; die hole diameter 4.0 mm (100×60 mm; 350 mm inside diameter)), cooling (UAS-Muyang, Model: SLNF14X14A; Counter flow) to +5°C ambient temperature, and then placed in

the 500 kg batch vacuum coater (UAS-Muyang, Model: SPYZ120). During vacuum coating 4% canola oil was added and mixed without (VC-) and with (VC+) vacuum coating. For VC-, oil was injected and mixed for one min before discharging for packaging. For VC+, pressure into the coater was lowered to 0.3 bar (0.03 MPa) and liquid was injected with constant mixing. Following oil addition, mixing was done for one min, while still maintaining vacuum. Then the vacuum pressure was released to allow air to go back inside the coater and move oil/enzyme further into pores. After internal pressure returned back to atmospheric pressure, the pellets were discharged for packaging.

For the second batch of four diets (Table 4.2; Diet No. 5-8), enzyme was added into the mixer and mixed with all ingredients before conditioning and pelleting (PreE+). Then, the diets were conditioned (65 or 95°C), pelleted and post-pellet oil application (VC- and VC+) was done as described above.

The third batch of four diets (Table 4.2; Diet No. 9-12) were produced by mixing the enzyme (inclusion level in oil was calculated to supply same enzyme activity as for Diet No. 5-8) in oil before post-pellet coating with VC- or VC+. Pelleting and coating were done using the same procedures as described above, except the enzyme was applied post-pelleting with oil.

4.3.3 Pellet durability

Pellet durability index (PDI) of pellets was calculated using a Holmen (Borregaard, UK Ltd. LT 218) durability tester, where 100 g of screened pellets were circulated pneumatically inside a chamber with a 2 mm screen for 30 s. The PDI was calculated as the proportion of pellets that did not pass through the screen.

4.3.4 Enzyme analysis

Enzyme activity of the feed samples was analysed using a Megazyme xylanase assay kit (Megazyme International Ireland Ltd, Bray, Ireland). Feed samples (~ 50g) were milled through a 0.5 mm screen (Rheinische Strabe 36 D-42781 Haan, Germany) and mixed thoroughly. Then 0.5 g of each sample was weighed (in quadruplicate) into test tubes and 5 mL of 0.1 M acetic acid was added. In two of the tubes, 0.2 mL of distilled water was added and in the remaining two tubes 0.2 mL of control xylanase solution (*Trichoderma sps*. Xylanase; 740 mU/0.2 ml) was added. The tubes were then incubated at room temperature for 20 min, stirring occasionally. The tubes were centrifuged (Avanti J-E centrifuge, Beckman Coulter Inc. Brea, CA, USA) at 1500x*g* for 10 min and supernatants were used for enzyme activity assay. Then 0.5 mL aliquot of supernatant solution (in duplicate) was transferred to glass tubes at room temperature. Then, a Xylazyme AX tablet was added to each tube before incubating in water bath at 50°C for 30 min. After incubation, 5 mL of Trizma base solution was added, stirred vigorously, and stored at room temperature for 5 min. Then tubes were stirred and the slurry was filtered through Whatman No. 1 (9 cm) filter paper. Absorbance of the filtrates was measured at 590 nm against a reaction blank. The reaction blank was prepared by adding Trizma Base solution (5 mL) to the feed extract (0.5 mL), followed by a Xylazyme AX tablet. The slurry was stirred and stored at room temperature for 5 min before filtering through Whatman No. 1 filter paper. The level of xylanase in the feed sample was calculated as follows:

$$\text{Activity in feed sample (0.5 gm)} = \text{Added activity} \times \frac{\text{SA}}{\text{TA}-\text{SA}}$$

Where,

- 'Added activity' is the amount of xylanase added to the feed sample slurry at the time of assay; 740 mU in the control xylanase solution (0.2 mL).

- ‘SA’ is reaction absorbance obtained for extracts of the feed sample to which no control xylanase was added.
- ‘TA’ is the total absorbance i.e. the absorbance of extracts of the feed sample to which the control xylanase was added.

4.3.5 Extract viscosity measurement of feed

The viscosity of the feed was determined as described by Scoles, et al., (1993). Diets were ground through a 1 mm screen and 0.5 g was weighed into 12×75 mm glass tubes. Then 1 mL of 0.1 N sodium acetate buffer (pH 5.0) was added and incubated for 30 min in a shaking water bath at 40°C. Contents of tube was poured into a 1.8 mL microcentrifuge tube and centrifuged (Beckman Microfuge E^{TM} ,Brea, CA, USA) for 5 min at 15,000 rpm (18,900x*g*). The supernatant (0.5 ml) was used to determine viscosity using a Brookfield cone-plate viscometer (Model LVDV-111, Brookfield Engineering Laboratories Inc. Middleborough, MA, USA) maintained at 40°C (40 rpm; shear rate 300 s^{-1}).

4.3.6 Birds and housing

A total of 400 day-old Ross 308 male broilers were randomly kept in battery cages until 10d. At 10d, groups of five birds were weighed and put in 72 respective cages (29 cm (height) × 48 cm (wide) × 83 cm (length); providing 800 cm^2/bird). Each of the 12 diet treatments were randomly assigned to six cages of five male broilers per cage. From 21 to 35d, the 15 remaining birds from each treatment were randomly assigned in groups of five birds into three cages (38 cm (height) × 66 cm (wide) × 69 cm (length); providing 900 cm^2/bird cages). A total of 36 cages were used in the grow/finish period for the 12 diets. Birds were provided free access to feed and water. Brooding temperature was 34°C at 0d and was then gradually reduced to 20°C at 21d.

The light in the room was 30-40 lux from 0-7 days (23:1; light: dark hr) and 10-20 lux from 7d onwards (20:4; light: dark hr).

4.3.7 Performance

At 21d, the average body weight of the birds (ABW) and the weight gain from 10d (BWG) were determined. The feed intake (gram/bird/day) of experimental starter diets from 10 to 21d was determined. The feed conversion ratio (feed: gain) for the 10-21d period was then calculated after correcting for mortality. Similar measurements were made for each cage for the grow/finish period (21 to 35d).

4.3.8 Bird sampling and measurements

At 21 and 35d, two birds from each cage were killed (cervical dislocation) and the gut segments were identified and measured. The weight of heart, liver, empty and fat-free proventriculus, and gizzard were recorded. Length of intestine segments (duodenum, jejunum, ileum and caeca) were measured and empty weights were recorded. The digesta from the duodenal loop was pooled for the two birds per cage and sampled for viscosity measurements in duplicate as described previously by Scoles et al. (1993).

4.3.9 Chemical analysis

Excreta samples of starter and grow/finish birds were collected on 17 and 18d, and on 30 and 31d, respectively, for determining apparent metabolizable energy (AME, kcal/kg diet) and nitrogen retention (NR; %). A clear plastic sheet was spread beneath each cage two times each day (morning and afternoon) to collect four samples of excreta from each cage. The excreta samples collected were oven dried for 72 h at 55°C. After drying, samples from each cage and diet phase were pooled together for analysis. Both diet and excreta were ground using a Retsch grinder with a 1.0 mm screen (ZM-100, Rheinische Strabe 36 D-42781, Haan, Germany). All

analyses were done in duplicate. Dry matter was determined by drying in a forced-air oven at 135°C for two h (AOAC No.; 15thed, 1990). Crude protein (N × 6.25) was determined using a Leco analyzer (Model FP-528L, Leco Corp., St. Joseph, MI, USA) with EDTA as a standard, according to the procedure described in AOAC (1995). Gross energy was determined by adiabatic oxygen bomb calorimeter (PARR, 6400, Moline, IL, USA), using benzoic acid as a standard. Celite^{TM}585 (Acros Organic, Fisher Scientific, Hampton, NH, USA), an acid insoluble ash marker (AIA), was analyzed using a modified procedure from Vogtmann et al. (1975). To measure AIA, 1-2 g of ground diet or excreta were weighed into 16×125 mm glass tubes (VWR North America, West Chester, PA, USA). The tubes were heated at 500°C for 24 h. The ash samples were then mixed with 5 mL of 4 N HCl and then heated (Isotemp, Fisher Scientific, Hampton, NH, USA) for 1 h at 120°C. Samples were then centrifuged (Avanti J-E Centrifuge, Beckman Coulter Inc., Brea, CA, USA) at 2500 rpm (1526x*g*) for 10 min. The supernatants were carefully removed using a vacuum siphon and samples were washed twice with 5 mL water and then dried at 80°C overnight. These dried samples were further kilned at 500°C overnight. The calculation of apparent metabolisable energy and nitrogen retention were determined as described by Scott and Hall (1998). The formulas used for the calculation are:

$$\text{AME (kcal/kg of diet)} = GE_{diet} - [GE_{excreta} \times (Marker_{diet}/Marker_{excreta})]$$

$$NR = 100 - [100 \times (\%Maker_{diet}/\%Marker_{excreta}) \times (\%N_{excreta}/N_{diet})]$$

4.3.10 Statistical Analysis

Statistical analysis was completed using a 3×2×2 factorial design for 3 enzyme addition methods (without enzyme, pre-pellet enzyme addition and post-pellet enzyme addition); 2 conditioning temperatures (65 and 95°C); and 2 vacuum coating (with and without vacuum) treatments.

Analysis was carried out using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc, 1996) and significant treatment means were separated using Turkey's Test. Significance level was set at $P \leq 0.05$ and trends set at $P \leq 0.1$.

4.4 Results

4.4.1 Pellet parameters

The average values for diet extract viscosity, enzyme activity and pellet durability index (PDI) of pellets are presented in Table 4.3. There was a 3-way interaction effect on pellet extract viscosity for starter (P=0.05; Figure 4.1) and grow/finish (P=0.07; Figure 4.2) diets. The 3-way interaction indicates that without enzyme addition there was an increase in extract viscosity with lower CT for diets with or without vacuum coating, whereas, these differences were not significant for the pre- or post-pellet addition of enzymes with or without VC. In the case of grow/finish pellets (Figure 4.2), the 3-way interaction shows that extract viscosity was higher for CT of 65°C as compared to 95°C when enzyme was not added. Whereas, when enzyme was added pre-pelleting, 95°C CT had a higher viscosity than 65°C.

In the case of starter diets, enzyme activity was affected by all main effects, however, there was a 2-way interaction (P=0.04) between EAM and CM (Figure 4.3). The interaction indicates that post-pellet vacuum coating (PosE+_VC+) of enzyme retained more activity in comparison to post-pellet coating without vacuum (PosE+_VC-), but there was no difference between CM for E- or PreE+ treatments. In the grower diets, there was a 2-way interaction (P=0.02) between EAM and CT for enzyme activity (Figure 4.4). The interaction suggests that with low CT, the enzyme activity was only higher than for high CT when enzyme was added pre-pelleting.

The PDI (Table 4.3) of starter and grower pellets was affected by CT. In both cases, a high CT improved PDI of the pellets. In starter pellets, vacuum coating significantly reduced PDI while this was not evident in grower pellets.

Table 4.3 Effect of enzyme, temperature and coating methods on diet extract viscosity (n=24), enzyme activity (n=48) and pellet durability index (n=36) of starter and grow/finish pellets.

		Starter pellet			Grow/Finish pellet		
Item	**Level[4]**	**Extract Viscosity (cP)[1]**	**Enzyme activity (unit/kg)[2]**	**PDI (%)[3]**	**Extract Viscosity (cP)**	**Enzyme activity (unit/kg)**	**PDI (%)**
Enzyme addition method (EAM)	E-	11.8[a]	49.3[c]	90.0	11.7[a]	65.9[c]	90.9
	PreE+	9.2[b]	988.3[b]	89.9	10.2[b]	1240.6[b]	91.4
	PosE+	5.9[c]	1900.8[a]	90.2	5.6[c]	2310.1[a]	90.3
SEM[5]		*0.22*	*18.29*	*0.44*	*0.16*	*27.73*	*0.68*
Conditioning temperature (CT)	65	9.8[a]	1001.6[a]	88.2[b]	9.5[a]	1225.0	88.5[b]
	95	8.2[b]	957.3[b]	91.9[a]	8.9[b]	1186.1	93.3[a]
SEM		*0.18*	*14.94*	*0.36*	*0.13*	*22.64*	*0.56*
Coating method (CM)	VC-	9.1	952.3[b]	90.6[a]	9.7[a]	1182.7	90.9
	VC+	8.9	1006.6[a]	89.5[b]	8.8[b]	1228.4	90.9
SEM		*0.18*	*14.94*	*0.36*	*0.13*	*22.64*	*0.56*
P-values, main effects and interaction							
EAM		*<0.01*	*<0.01*	*NS*	*<0.01*	*<0.01*	*NS*
CT		*<0.01*	*0.04*	*<0.01*	*0.01*	*NS*	*<0.01*
CM		*NS[6]*	*0.01*	*0.05*	*<0.01*	*NS*	*NS*
EAM×CT		*<0.01*	*NS*	*NS*	*<0.01*	*0.02*	*NS*
EAM×CM		*NS*	*0.04*	*NS*	*<0.01*	*NS*	*NS*
CT×CM		*0.01*	*NS*	*NS*	*0.08*	*NS*	*NS*
EAM×CT×CM		*0.05*	*NS*	*NS*	*0.07*	*NS*	*NS*

[1]Viscosity is measured in centipoise (1 cP=0.01 g/cm/s)

[2]Enzyme activity is measured as xylanase unit as described in materials and methods

[3]Pellet durability index

[4]EAM; enzyme addition method, (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme); CT; conditioning temperature (65, low temperature (65°C); 95, high temperature (95°C)); CM coating method (VC-, without vacuum; VC+, with vacuum).

[5] Standard error of mean

[6]Not significant

[a-c] Means within columns for each item with no common superscripts are significantly different (P≤ 0.05)

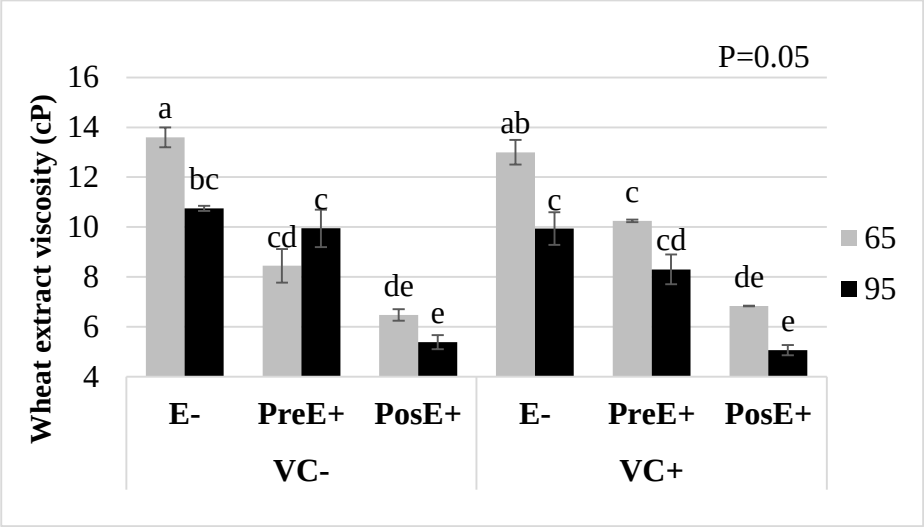

Figure 4.1 Interaction of enzyme addition method, conditioning temperature and coating method on wheat extract viscosity of broiler starter pellets, n=2 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-e) are significantly different.

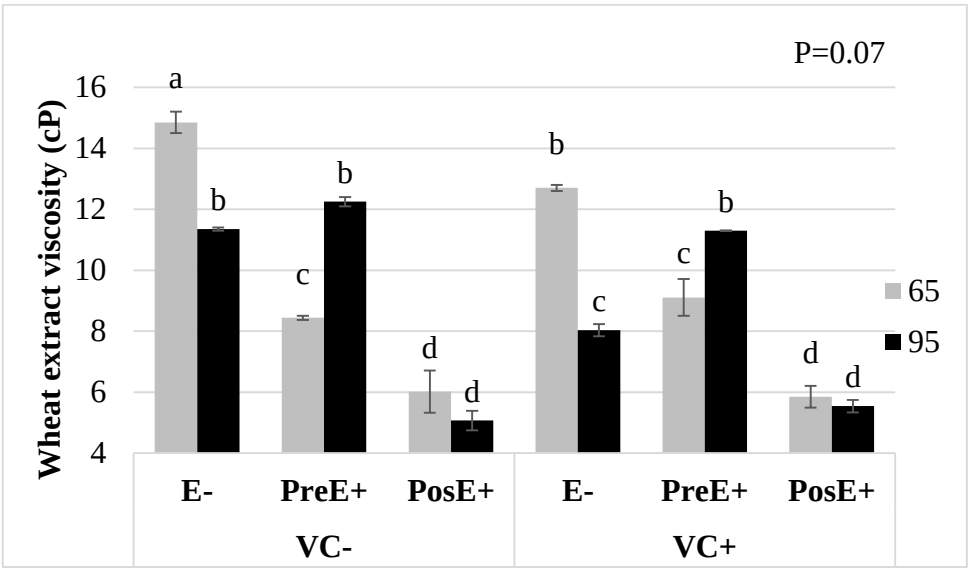

Figure 4.2 Interaction of enzyme addition method, conditioning temperature and coating method on wheat extract viscosity of broiler grow/finish pellets, n=2 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-d) are significantly different.

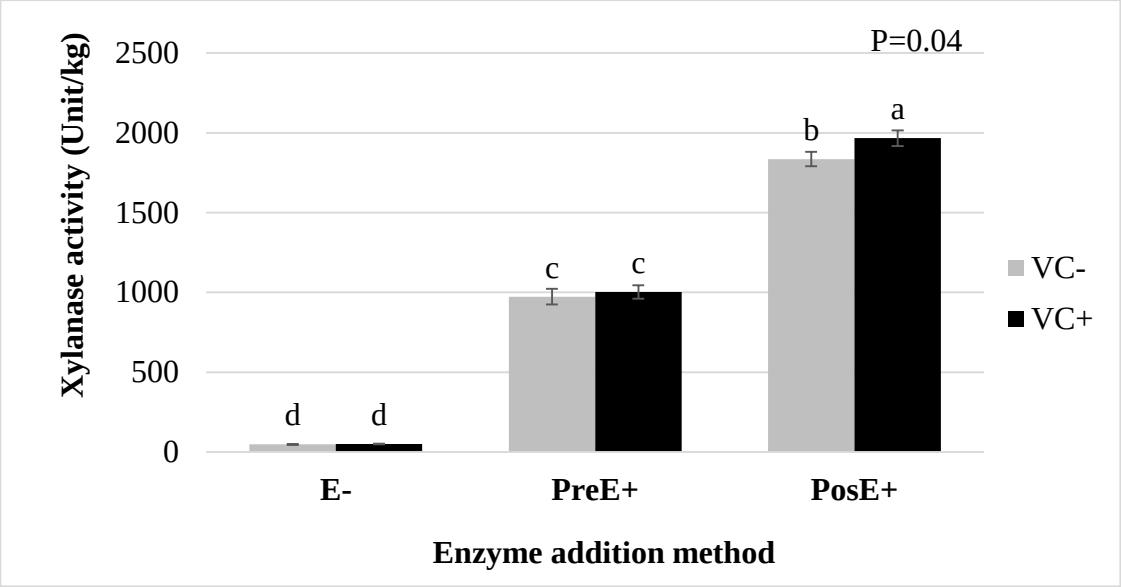

Figure 4.3 Interaction of enzyme addition method and coating method on enzyme activity of starter pellets, n=8 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme;VC-, without vacuum; VC+, with vacuum) Bars without common letters (a-d) are significantly different.

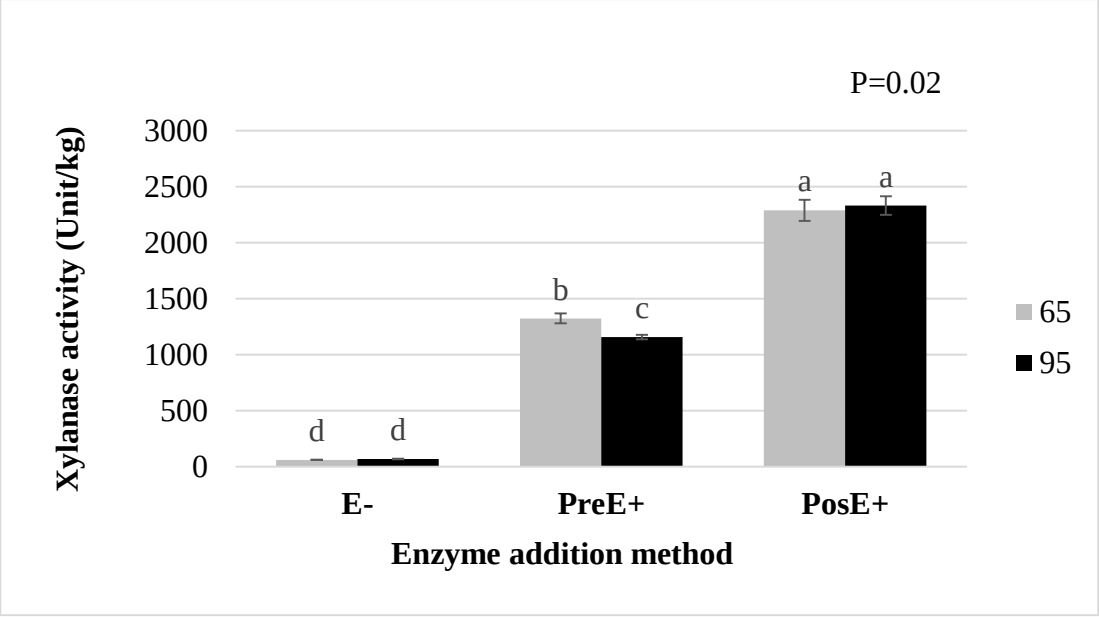

Figure 4.4 Interaction of enzyme addition methods and conditioning temperature on enzyme activity of grow/finish pellets, n=8 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-d) are significantly different.

4.4.2 Performance

The average body weight (ABW), body weight gain (BWG), feed intake (FI) and mortality adjusted feed conversion ratio (FCR) are presented in Table 4.4 for 10 to 21d and Table 4.5 for 21 to 35d. Overall mortality was 2.5% from 10 to 21d and 8.3% from 21 to 35d. Mortality was not associated with any of the treatments. The average 21d body weight for all main effects was slightly above that provided by Aviagen (2014) broiler performance guidelines (959 g). There was a significant 3-way interaction (P=0.03) of enzyme addition methods (EAM), conditioning temperature (CT) and coating methods (CM) on average body weight at 21d (Figure 4.5). The interaction indicates that the average body weight was higher when birds were fed with diets conditioned at 95°C with PreE+ and VC- in comparison to E-_95_VC- but there was no difference from other treatments. However, at 35d, there was only an effect of EAM on ABW, indicating an overall positive effect of enzyme on ABW.

For body weight gain (BWG), there was 3-way interaction (P<0.01) of EAM, CT and CM during the 10-21d period and it was similar to the findings for ABW. For the period 21-35d, the BWG tends (P<0.08) to be affected by EAM. Enzyme addition, either pre-pellet mixing (PreE+) or post-pellet coating (PosE+), improved the ABW of birds in comparison to without enzyme addition (E-) in both phases. There was no difference in ABW for PreE+ and PosE+. There were no significant effects of treatments or interactions among treatments for feed intake in 10-21d period. However, in the grow/finish phase, 65°C CT compared to 95°C CT increased feed intake.

There were significant main effects for EAM, CT and their interaction between EAM and CT on feed conversion ratio (FCR) for 10-21d (Figure 4.6). The interaction indicates that there were only significant differences in FCR when no enzyme was present and the FCR was lower

when conditioned at 95°C as compared to 65°C. There were no effects of CT when enzyme was added to the diets either PreE+ or PosE+. In the grow/finish phase, FCR was affected by both EAM (P=0.03) and CT (P=0.04); there were no interactions for FCR from 21-35d. The FCR in the grow/finish phase was highest for E-, but not different from PreE+, the latter not being different from PosE+. Vacuum coating did not have any effect on the FCR.

4.4.3 Digestibility

The digestibility data for starter and grow/finish diets were presented in Tables 4.4 and 4.5. The apparent metabolisable energy (AME) of starter diets demonstrated an interaction between EAM and CT (P<0.01) (Figure 4.7). The two-way interaction indicates that there were significantly higher AME levels with 95°C as compared to 65°C CT when no enzyme (E-) and post-pellet enzyme (PosE+) treatments were applied; whereas, with pre-pellet enzyme application (PreE+), there was no difference between the two CT.

There was a 2-way interaction (P<0.01) between CM and CT for nitrogen retention (NR) of starter diets (Figure 4.8). The interaction suggests there was no difference in NR between two CT for both CM. However, NR for 65_VC+ was higher than 65_VC- but not different from other treatment combination. In the case of grower diets (Table 4.5), there was an effect of EAM (P=0.04) and CT (P=0.01) on AME. AME for PosE+ was highest and E- was lowest, but both were not different from PreE+.

Table 4.4 Effect of enzyme, temperature and coating methods on starter broilers (10-21d) performance (average body weight, body weight gain, feed intake, FCR) and digestibility (apparent metabolizable energy (AME; kcal/kg diet) and nitrogen retention (% NR))

		Starter (10 -21 d)					
Item	Level[1]*(n)*	ABW (g)[2]	BWG (g)[3]	FI (g/b/d)[4]	FCR (g/g)[5]	AME[6] (Kcal/kg)	NR[7] (%)
Enzyme addition method (EAM)	E- *(24)*	983[b]	741.1[b]	94.0	1.52[a]	2773[b]	57.1
	PreE+*(24)*	1027[a]	787.1[a]	96.0	1.47[ab]	2886 [ab]	57.6
	PosE+*(24)*	1026[a]	783.0[a]	93.6	1.44[b]	2915[a]	57.6
SEM[8]		*11.3*	*11.14*	*1.34*	*0.020*	*36.9*	*0.86*
Conditioning temperature (CT)	65*(36)*	1006	764.2	95.7	1.51[a]	2784[b]	58.0
	95*(36)*	1018	776.6	93.3	1.44[b]	2933[a]	57.8
SEM		*9.2*	*9.10*	*1.09*	*0.016*	*30.1*	*0.70*
Coating method (CM)	VC-*(36)*	1012	769.5	94.6	1.48	2836	57.3
	VC+*(36)*	1012	771.2	94.7	1.47	2881	58.4
SEM		*9.2*	*9.10*	*1.09*	*0.016*	*30.1*	*0.70*
P-values, main effects and interaction	*df*						
EAM	*2*	*0.01*	*<0.01*	*NS*	*0.03*	*0.02*	*NS*
CT	*1*	*NS*[9]	*NS*	*NS*	*<0.01*	*<0.01*	*NS*
CM	*1*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT	*2*	*NS*	*NS*	*NS*	*0.03*	*<0.01*	*NS*
EAM×CM	*2*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
CT×CM	*1*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.01*
EAM×CT×CM	*2*	*0.03*	*0.03*	*NS*	*NS*	*NS*	*NS*

[1]EAM; enzyme addition method, (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme); CT; conditioning temperature (65, low temperature (65°C); 95, high temperature (95°C)); CM coating method (VC-, without vacuum; VC+, with vacuum); n; n values
[2]Average bodyweight in grams, [3]Body weight gain in grams, [4]Feed intake in gram per bird per day, [5]Feed conversion ratio adjusted for mortality
[6]Apparent metabolisable energy (kcal/kg), [7]Nitrogen retention (%),
[8]Standard error of mean
[9]Not significant
[a-b] Means within columns for each item with no common superscripts are significantly different (P≤ 0.05)

Table 4.5 Effect of enzyme, temperature and coating methods on grower broilers (21-35d) performance (average body weight, body weight gain, feed intake, FCR) and digestibility (apparent metabolizable energy (AME; kcal/kg diet) and nitrogen retention (% NR))

		Growers (21-35 d)					
Item	Level[1]*(n)*	ABW (g)[2]	BWG (g)[3]	FI (g/b/d)[4]	FCR (g/g)[5]	AME[6]	NR[7]
Enzyme addition method (EAM)	E- *(12)*	2190[b]	1175	153.0	2.01[a]	2863[b]	54.2
	PreE+*(12)*	2302[a]	1254	148.5	1.92[ab]	2961[ab]	54.0
	PosE+*(12)*	2314[a]	1263	150.0	1.83[b]	3061[a]	50.2
SEM[8]		*23.2*	*29.8*	*4.29*	*0.434*	*51.1*	*1.63*
Conditioning temperature (CT)	65 *(18)*	2268	1241	156.9[a]	1.97[a]	2883[b]	52.3
	95*(18)*	2269	1220	144.1[b]	1.87[b]	3041[a]	53.3
SEM		*18.9*	*24.3*	*3.50*	*0.035*	*41.7*	*1.33*
Coating method (CM)	VC-*(18)*	2254	1213	151.9	1.93	2917	53.6
	VC+*(18)*	2284	1249	149.1	1.91	3007	52.0
SEM		*18.9*	*24.3*	*3.50*	*0.035*	*41.7*	*1.33*
P-values, main effects and interaction	*df*						
EAM	*2*	*<0.01*	*0.08*	*NS*	*0.03*	*0.04*	*NS*
CT	*1*	*NS*[9]	*NS*	*0.02*	*0.04*	*0.01*	*NS*
CM	*1*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT	*2*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CM	*2*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
CT×CM	*1*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT×CM	*2*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

[1]EAM; enzyme addition method, (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme); CT; conditioning temperature (65, low temperature (65°C); 95, high temperature (95°C)); CM coating method (VC-, without vacuum; VC+, with vacuum); [2]Average bodyweight in grams, [3]Body weight gain in grams, [4]Feed intake in gram per bird per day, [5]Feed conversion ratio adjusted for mortality

[6]Apparent metabolisable energy (kcal/kg), [7]Nitrogen retention (%),

[8]Standard error of mean

[9]Not significant

[a-b] Means within columns for each item with no common superscripts are significantly different (P≤ 0.05)

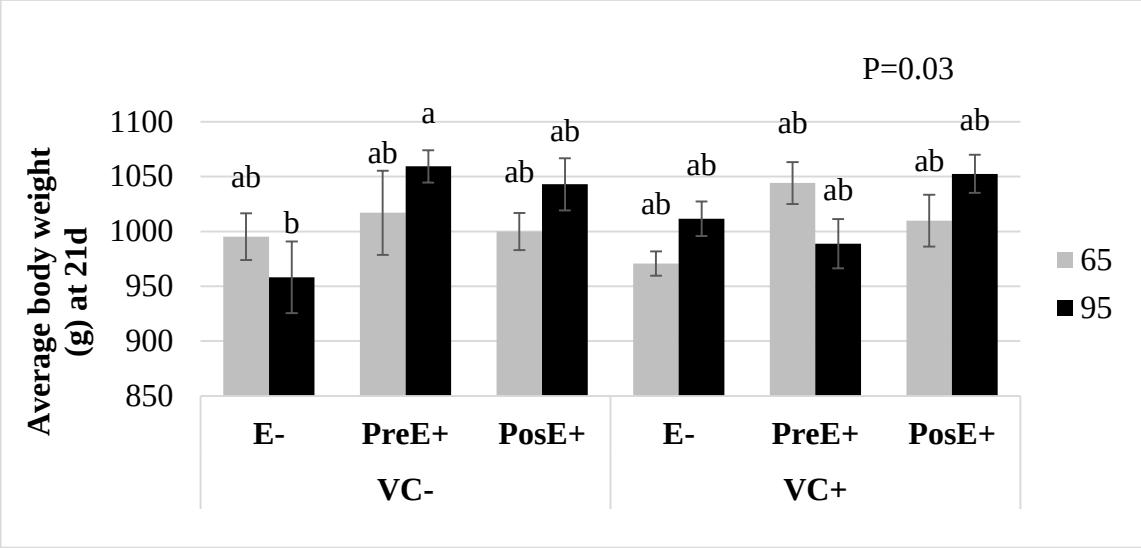

Figure 4.5 Interaction of enzyme addition method, conditioning temperature and coating method on average body weight of broilers at 21d, n=6 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-b) are significantly different.

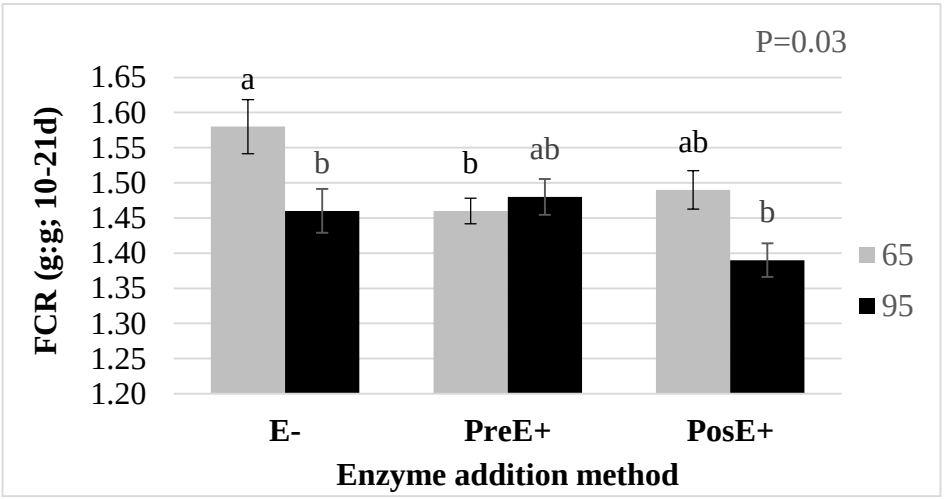

Figure 4.6 Interaction of enzyme addition method and conditioning temperature on feed conversion ratio (FCR) of 10-21d broilers, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-b) are significantly different.

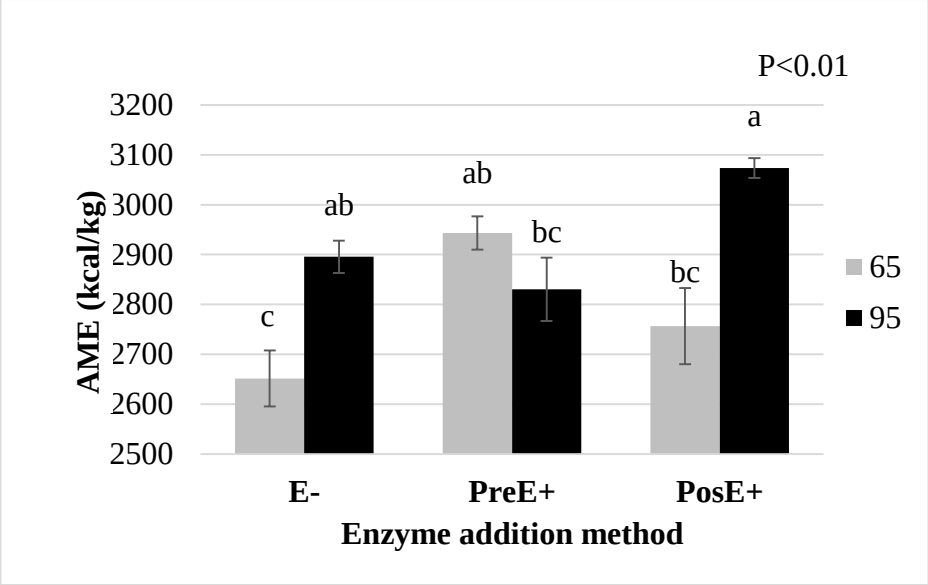

Figure 4.7 Interaction of enzyme addition method and conditioning temperature on apparent metabolisable energy (AME) of 21d broilers, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-c) are significantly different.

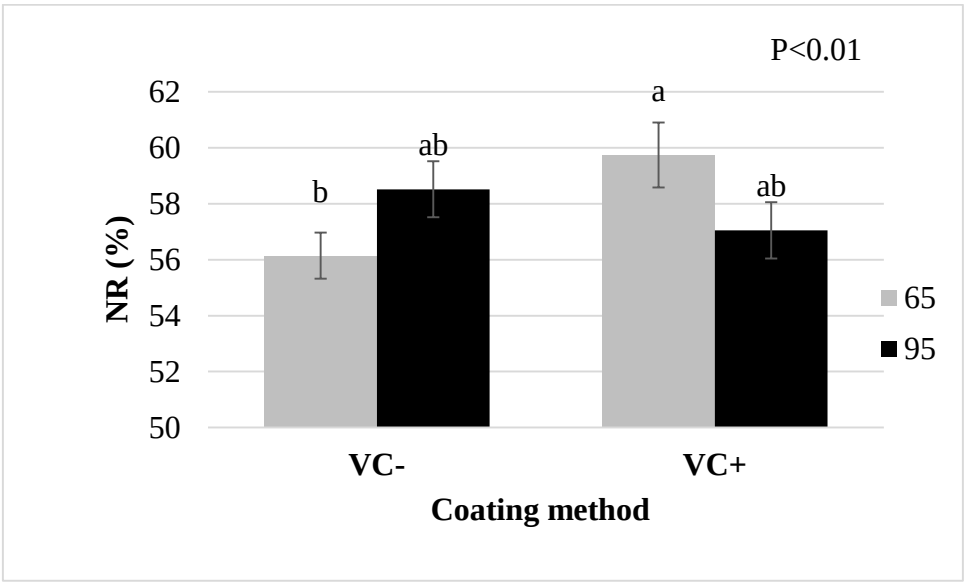

Figure 4.8 Interaction of conditioning temperature and coating method on nitrogen retention (NR) of 21d broilers, n=18 (65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum) Bars without common letters (a-b) are significantly different.

4.4.4 Digestive tract development and digesta viscosity

The values for relative length and weight of digestive tract and digesta viscosity for birds sampled at 21 and 35d, respectively, are presented in Table 4.6 and 4.7. For the starter diets, there was a higher relative gizzard weight when CT was 65°C as compared to 95°C. For relative proventriculus weight, there were significant main effects for CT and CM. The proventriculus was larger at 65 as compared to 95°C. The relative proventriculus weight was higher ($P<0.05$) for VC+ as compared to VC-. There were no differences in these two organs when measured at 35d (data not shown).

There were only significant effects for duodenum and ileum relative weights of the intestinal segments at 21d after feeding starter diets from 10-21d (Table 4.6). Relative duodenum weight was lower for PreE+ as compared to PosE+, and both were not significantly different from E-. The 65°C CT in the starter diets resulted in a relative heavier ileum as compared to 95°C. Similarly, birds fed with VC- diets had heavier ileum weights compared to VC+, whereas during the grow/finish phase (21-35d; Table 4.7), there were only significant effects on relative jejunum and caeca weights. In both cases, these segments were lighter with 95°C as compared to 65°C CT. In this phase, the relative weight of the jejunum was higher in PosE+ as compared to PreE+ but not different from E-. There was a trend ($P=0.06$) for interaction of EAM and CT for caeca weight during the 21-35d period (Figure 4.9). The interaction shows relative caeca weight was highest with PosE+_65, which is not different from the other treatment combinations with the exception of E-_95.

With respect to relative length of intestinal segments, there were significant effects for the starter (10-21d; Table 4.6) and grow/finish (21-35d; Table 4.7) phases. For the duodenum (21d), the main effects of EAM and CM were significant. The relative length of the duodenum was

greater for E- as compared to PreE+ and both were not different from the intermediate value for PosE+. There was a relatively longer duodenum lengths for VC- as compared to VC+. There were no treatments effects on relative duodenum length at 35d (data not shown).

For relative jejunum length, there was a main effect of EAM at 21d, and this was similar to that recorded for relative duodenum length (E->PosE+>PreE+) with a significant difference between E- and PreE+ only. Similarly at 21d, there was a trend (P=0.09) for a longer jejunum with VC- as compared to VC+. At 35d, there was only a significant main effect of CT on relative jejunum length and it was longest for 65 as compared to 95°C.

The relative ileum length measured at 21d was significantly affected by the main effects VC and a 3-way interaction (Figure 4.10). The 3-way interaction indicates that relative ileum length was longest for E-_95_VC-, however, this was not different from any other treatment combination except PreE+_65_VC+. Similarly at 35d, the relative ileum length was affected by main effects CT and a 2-way interaction (EAM x CT; Figure 4.11). The interaction indicates that relative ileum length was longest in birds fed with PosE+_65, which was not different from E-_95 but higher than for other treatment combinations. The relative ileum lengths for birds fed with E-_95 diets were not different from any of the other treatment combinations.

Relative caeca length at 21 d (Table 4.6) was significantly impacted by CM and a trend (P=0.09) for a 3-way interaction (Figure 4.12). The 3-way interaction indicates that relative caeca length was significantly higher for Pre+_65 for VC- than VC+ but both treatment combination were not different from the other treatment combinations. Whereas the relative caeca length at 35d was significantly greater at 65°C as compared to 95°C, and there was a trend for a 2-way interaction between EAM and CT (P=0.09; Figure 4.13). The 2-way interaction

shows relative caeca length was longer for 65°C compared to 95°C for both E- and PosE+, however not different between both CT for PreE+.

There was significant 3-way significant interaction effect (P=0.04) on digesta viscosity in the starter phase (Figure 4.14). The 3-way interaction for digesta viscosity at 21d indicates that there was no difference in digesta viscosity between two CT for all EAM and CM. Digesta viscosity of E- at 65°C CT and VC+ was significantly higher than PosE+ with both CT and CM and PreE+ with VC+, but not different from all other treatment combinations.

Table 4.6 Influence of enzyme addition method, conditioning temperature and coating methods on digestive tract development (relative length and weight) and digesta viscosity of broilers at 21 d of age[1, 2]

		Relative weight (g /kg body weight)				Relative length (cm/kg body weight)				Dig. Visc (cP)[7]
Item	Level[3]	Gizzard	Prov[4]	Duo[5]	Ileum	Duo	Jej[6]	Ileum	Caeca	
Enzyme addition method (EAM)	E-	14.3	5.7	8.0^{ab}	13.6	25.3^{a}	63.9^{a}	60.6	20.9	4.35^{a}
	PreE+	15.2	5.3	7.2^{b}	13.0	23.8^{b}	57.9^{b}	58.3	21.4	3.47^{b}
	PosE+	14.9	5.6	8.2^{a}	12.9	25.0^{ab}	60.1^{ab}	60.4	21.9	2.73^{c}
SEM[8]		*0.44*	*0.18*	*0.24*	*0.33*	*0.43*	*1.23*	*1.4*	*0.52*	*0.181*
Conditioning temperature (CT)	65	15.7^{a}	5.9^{a}	7.7	13.8^{a}	25.0	60.4	59.4	21.4	3.79^{a}
	95	13.9^{b}	5.3^{b}	7.9	12.5^{b}	24.4	60.9	60.1	21.5	3.24^{b}
SEM		0.35	0.14	0.20	0.27	0.36	1.00	1.14	0.42	*0.148*
Coating method (CM)	VC-	15.1	5.3^{b}	8.0	13.7^{a}	25.5^{a}	61.8	62.4^{a}	22.3^{a}	3.45
	VC+	14.6	5.8^{a}	7.6	12.7^{b}	23.9^{b}	59.5	57.1^{b}	20.6^{b}	3.58
SEM		*0.35*	*0.14*	*0.20*	*0.27*	*0.36*	*1.00*	*1.14*	*0.42*	*0.148*
P-values, main effects and interaction										
EAM		*NS*[9]	*NS*	*0.01*	*NS*	*0.04*	*<0.01*	*NS*	*NS*	*<0.01*
CT		*<0.01*	*<0.01*	*NS*	*<0.01*	*NS*	*NS*	*NS*	*NS*	*0.01*
CM		*NS*	*0.02*	*NS*	*0.01*	*<0.01*	*0.09*	*<0.01*	*<0.01*	*NS*
EAM×CT		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.05*
EAM×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
CT×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.03*	*0.09*	*0.04*

[1]The values for the segments of digestive tract that was not affected by any variables are not shown in the table

[2]Each value represents the mean of 12 birds

[3]EAM; enzyme addition method, (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme); CT; conditioning temperature (65, low temperature (65°C); 95, high temperature (95°C)); CM, coating method (VC-, without vacuum; VC+, with vacuum); [4]Proventriculus, [5]Duodenum, [6]Jejunum,

[7] Digesta viscosity (duodenal contents) measured in centipoise (1 cP=0.01 g/cm/s), each value represents the mean of 12 birds

[8]Standard error of mean

[9]NS, non-significant

[a-b] Means within columns for each item with no common superscripts are significantly different (P≤ 0.05)

Table 4.7 Influence of enzyme addition method, conditioning temperature and coating methods on digestive tract development (relative length and weight) and digesta viscosity of broilers at 35 d of age[1, 2]

		Relative weight (g /kg body weight)		Relative length (cm/kg body weight)			Dig. Visc (cP)[4]
Item	Level[3]	Jejunum	Caeca	Jejunum	Ileum	Caeca	
Enzyme addition method (EAM)	E-	11.5[ab]	3.0	29.0	28.7	12.6	7.29[a]
	PreE+	11.0[b]	3.0	29.4	28.7	12.2	6.25[ab]
	PosE+	12.02[a]	3.2	30.6	30.7	13.0	4.00b
SEM[5]		*0.27*	*0.10*	*0.88*	*0.84*	*0.36*	*0.800*
Conditioning temperature (CT)	65	12.2[a]	3.2[a]	30.9[a]	31.0[a]	13.4[a]	6.62
	95	10.8[b]	3.0[b]	28.4[b]	27.7[b]	11.8[b]	5.08
SEM		0.22	0.09	0.72	0.69	0.29	0.654
Coating method (CM)	VC-	11.6	3.2	29.8	29.7	12.6	5.95
	VC+	11.3	3.0	29.5	29.0	12.6	5.74
SEM		*0.22*	*0.09*	*0.72*	*0.69*	*0.29*	*0.654*
P-values, main effects and interaction							
EAM		*0.03*	*NS*	*NS*	*NS*	*NS*	*0.02*
CT		*<0.01*	*0.03*	*0.02*	*<0.01*	*<0.01*	*NS*
CM		*NS*[6]	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT		*NS*	*0.06*	*NS*	*<0.01*	*0.09*	*NS*
EAM×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
CT×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
EAM×CT×CM		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

[1]Each value represents the mean of 6 birds

[2]The values for the segments of digestive tract that was not affected by any variables are not shown in the table

[3]EAM; enzyme addition method, (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme); CT; conditioning temperature (65, low temperature (65°C); 95, high temperature (95°C)); CM coating method (VC-, without vacuum; VC+, with vacuum); [4]Digesta viscosity (duodenal contents) measured in centipoise (1 cP=0.01 g/cm/s)

[5]Standard error of mean

[6]NS, non-significant

[a-b] Means within columns for each item with no common superscripts are significantly different (P≤ 0.05)

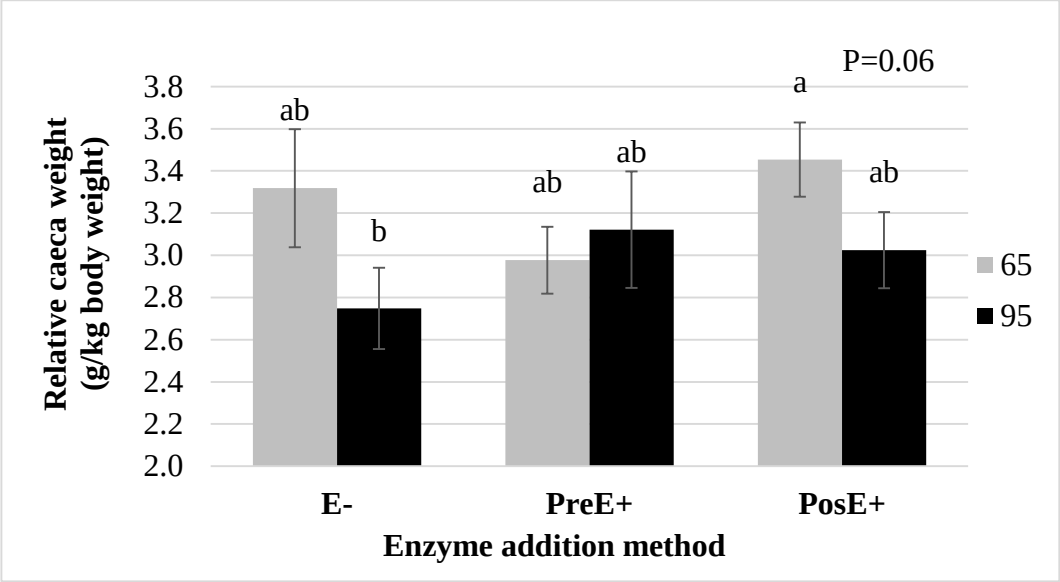

Figure 4.9 Interaction of enzyme addition method and conditioning temperature on relative caeca weight of 35d broilers, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-b) are significantly different.

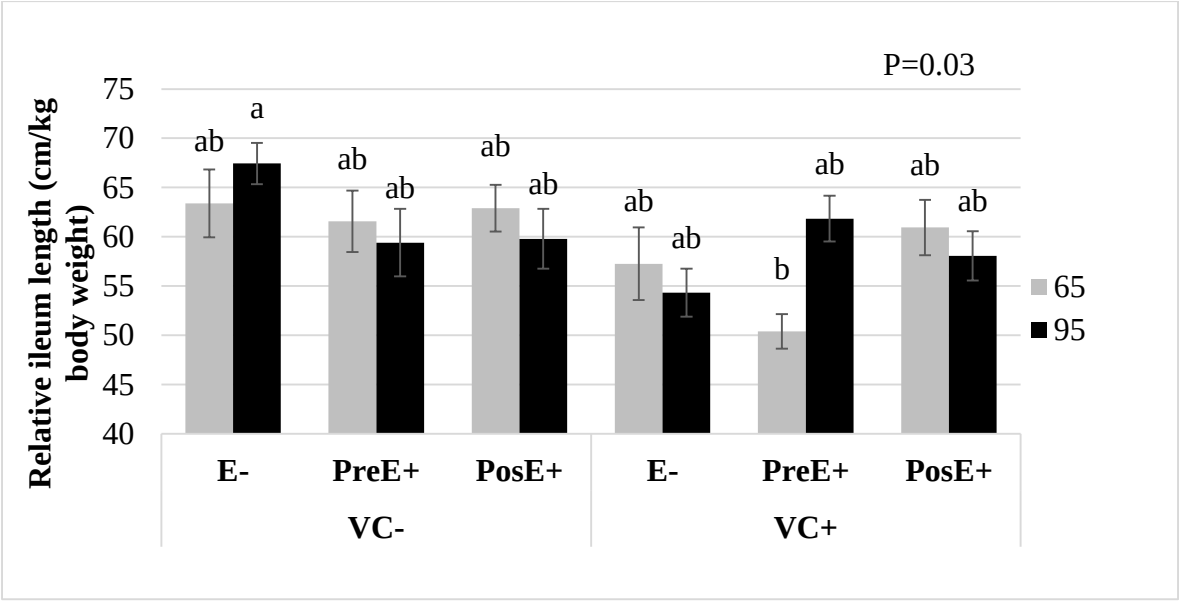

Figure 4.10 Interaction of enzyme addition method, conditioning temperature and coating method on relative ileum length of broilers at 21d, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-b) are significantly different.

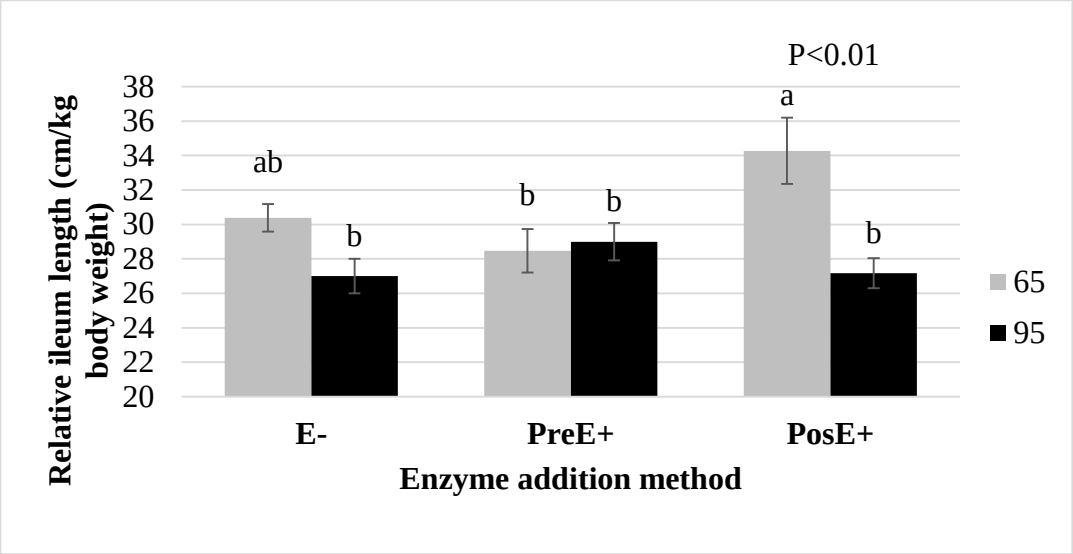

Figure 4.11 Interaction of enzyme addition method and conditioning temperature on relative ileum length of 35d broilers, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-b) are significantly different.

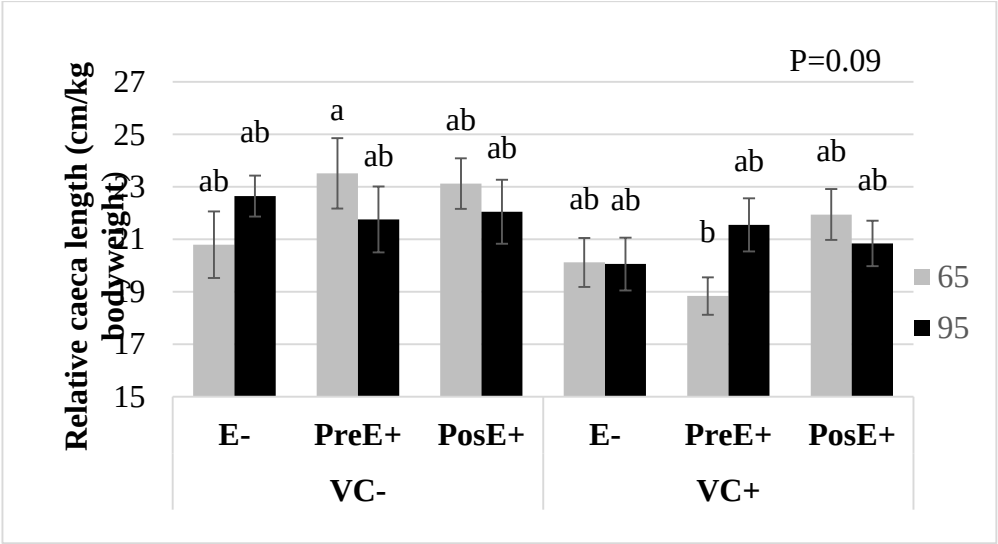

Figure 4.12 Interaction of enzyme addition method, conditioning temperature and coating method on relative caeca length of broilers at 21d, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-b) are significantly different.

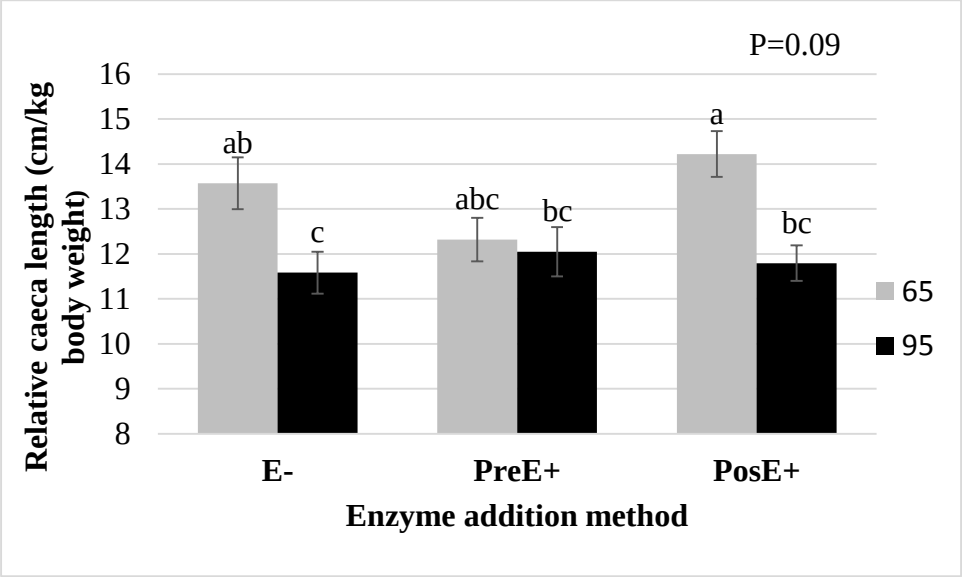

Figure 4.13 Interaction of enzyme addition method and conditioning temperature on relative caeca length of 35d broilers, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C)). Bars without common letters (a-c) are significantly different.

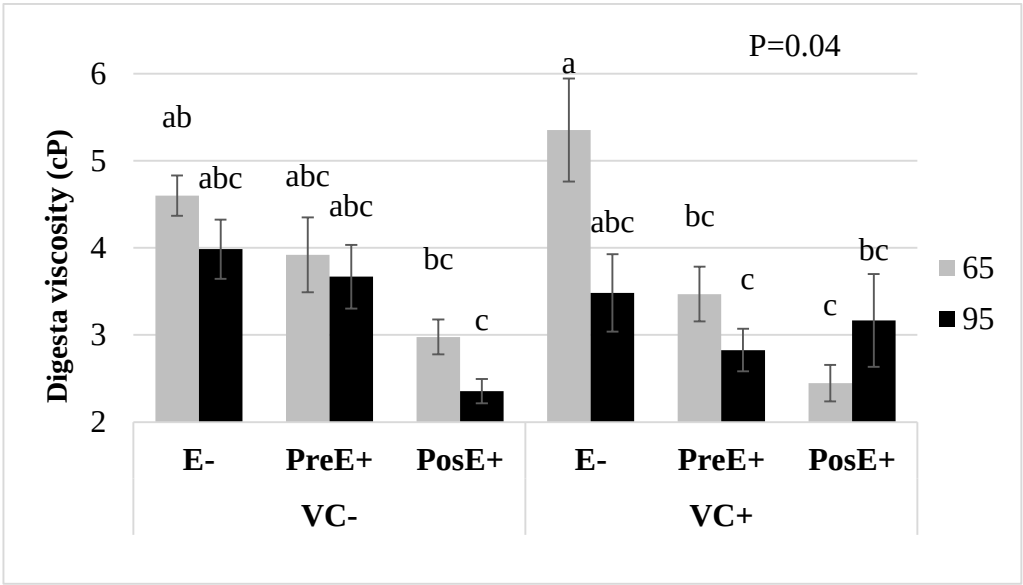

Figure 4.14 Interaction of enzyme addition method, conditioning temperature and coating method on digestive viscosity of broilers at 21d, n=12 (E-, no enzyme; PreE+, pre pellet addition of enzyme; PosE+, post-pellet addition of enzyme; 65, low temperature (65°C); 95, high temperature (95°C); VC-, without vacuum; VC+, with vacuum). Bars without common letters (a-c) are significantly different.

4.5 Discussion

Vacuum coating has traditionally been used for inclusion of high levels of fats in extruded products. Inclusion of heat-sensitive bioactives using vacuum coating technology is gaining popularity, but very limited work has been done to prove its applicability. There are very few studies that compare the effect of pre and post-pellet enzyme application on various pellet parameters and performance. In this study, when xylanase was added in the diet using various methods, vacuum coating of enzyme did not affect the diet extract viscosity. However, with post-pellet application (PosE+; either VC+ or VC-), the viscosity was significantly reduced when compared to diets without enzyme (E-) or pre-pellet addition of enzyme (PreE+).

This result is consistent when compared to retention of enzyme activity with various enzyme application methods. Xylanase enzyme activity was retained more when enzyme was added post-pelleting. The effect of conditioning temperature on diet viscosity was quite surprising. There is a general assumption that a high conditioning temperature increases the diet viscosity by releasing previously encapsulated NSP and increasing solubilisation of polysaccharides (Westerlund et al., 1989; Cowieson et al., 2005). In the present study, when enzyme was not added to the diet, the extract viscosity of diets conditioned at lower temperature (65°C) was higher in both starter and grow/finish pellets. However, when enzyme was added pre-pelleting to the mixture, higher conditioning temperature increased extract viscosity in grow/finish diets (Figure 4.2).

The reason for the decline in viscosity for low conditioning temperature in E- diets is not clearly understood. However, in grow/finish diets, with the increase in CT, the increase in viscosity of PreE+ diets might be due to decrease in enzyme activity associated with high temperature and less enzyme available for reduction of soluble NSP. This is supported by the

measurements of enzyme activity of PreE+ diets for CT of 65°C and 95°C (Figure 4.4), where 95°C has less enzyme activity retained. This result is consistent with the results obtained by Coweison et al. (2005). In their study, the in-vitro viscosity of finisher diets was reduced for pellets conditioned at 90°C in comparison to 85°C, but no effect of temperature was observed for starter pellets when conditioned at 85 or 90°C.

Vacuum coating did not affect the extract viscosity in either starter and grow/finish pellets. However, there was higher enzyme activity in the starter pellets with post-pellet vacuum coating (Figure 4.3). This can be attributed to reduction of enzyme activity losses in the fines. The inconsistent results of starter and grower pellets with coating method and temperature might be due to other variables associated with processing. The temperature, moisture and the vacuum coating parameters variation could result in different outcomes, and are difficult to control. Similarly, the lower activity in grower pellets for pre-pellet addition with high conditioning temperature might be due to destruction of enzyme at high temperature. Cowieson et al. (2005) recovered only 71 % of the activity after the supplemented feed was pelleted at 90°C. Similarly, in another study, there was 76% reduction of enzyme activity when feed was pelleted at 95°C with 55 sec of conditioning time (Silversides and Bedford, 1999). In the current study, there were only 4.4 % and 3.1 % reduction in enzyme activity, respectively, for starter and grower pellets when temperature was increased from 65°C to 95°C. However, when compared to pre and post-pellet application methods, there was 48 and 46% less enzyme activity with pre-pelleting regardless of conditioning temperature. Similarly, Inborr and Bedford (1994) found that activity of beta-glucanase was reduced by 36% when pelleted at 75°C and 85% when pelleted at 95°C in comparison to the enzyme-supplemented mash.

There are various factors affecting the thermostability of xylanase, such as, source of enzyme (Coral et al., 2002), method of enzyme production (Vahjen and Simon, 1999) and inclusion of heat stabilizing metals (Al^{3+}, Cu^{2+}, Hg^{2+}, Li^{+}, Mn^{2+}) to increase thermostability (Suleman et al., 2012). The industry is moving toward using enzyme cocktails (various sources of enzyme with different stabilities under different conditions, e.g., temperature, moisture, pH) or use of additives (e.g., heat stable coating) to increase efficacy after processing. However, the activity is typically reduced when the conditioning temperature is over 90°C, provided exposure time and moisture are accounted for.

In the current study, PDI was increased by 4.2% and 5.4%, respectively, for starter and grower pellets by increasing the conditioning temperature from 65°C to 95°C. Similar results were observed in other experiments where an increase in conditioning temperature significantly improved pellet durability (Abdollahi et al., 2010a; Lundblad et al., 2011). This might be because of increased steam injection, which subsequently increases starch gelatinisation and binding of the ingredients to form durable pellets. In the present study, PDI of starter pellets without vacuum application was slightly higher (90.6 vs 89.5 %) than with vacuum application. This result may be attributed to the extra handling of the pellets during the vacuum application. However, this was not evident in the case of grower pellets, indicating further studies need to be considered to fully understand the benefits of vacuum coating on functional and nutritional parameters.

In the current experiment, the average body weight of the birds at 21 and 35 d was found to be 1018 g and 2268 g respectively, which is acceptable when compared to Ross-308 performance goals (969 and 2283 g; Aviagen, 2014). The experiment demonstrated that addition of enzyme either pre- or post-pellet improved the average body weight of birds in both starter

and grower phase. The effect of NSPase enzyme addition on broiler performance has been well established. Cereal grains, such as wheat, barley and rye, have variable amounts of soluble and non-soluble NSP (Scott et al., 1998). The significant proportion of soluble arabinoxylan in wheat is of high molecular weight, which can increase the gut viscosity geometrically and impairs the diffusion of nutrients (Coweison et al., 2005). Fengler and Marquardt (1988) demonstrated that the major antinutritive factor in rye grain is highly viscous, water soluble, pentosan-rich carbohydrate. They also demonstrated that slight changes in the viscosity of digesta could result in dramatic changes on the diffusion of nutrients. The reduction in intestinal viscosity can improve the growth rate and feed efficiency of broiler chicks (Bedford and Classen, 1992; Engberg et al., 2004).

There was no significant difference in bodyweight between the two application methods, pre- and post-pellet enzyme addition. Similar results were observed in an experiment conducted by Marron et al. (2001) where no differences in broiler performance were observed with dry vs. post-pellet liquid spray applications of enzymes. However, for with and without enzyme supplementation, feed conversion was significantly improved by enzyme supplemented diets with no difference in bodyweight for either treatment. The current study demonstrated the interaction of enzyme and temperature on feed conversion of starter birds (Figure 4.6) and individual effects of enzyme and temperature on feed efficiency of grower birds. However, feed conversion for pre-pellet addition of enzyme was not different for E- or PosE+ in both starter and grower phases. Steenfeldt et al. (1998a) also demonstrated the improvement of feed efficiency with enzyme supplementation without any effect on body weight gain and feed intake. Similarly, Zhang et al. (2014) also showed no difference in feed intake with improvement in weight gain when wheat-based diets were supplemented with xylanase. Yang et al. (2008)

demonstrated that body weight gain and FCR were improved in broilers during the 8-21d period, but an effect on feed intake was not evident.

The improvement in broiler performance with enzyme supplementation can be accounted for reduced intestinal viscosity and the improvement of nutrient diffusion across the intestinal membrane. Maroon et al. (2001) observed a 6% improvement in FCR; that was associated with a 52% reduction in ileal digesta viscosity. In the present study, the duodenal digesta viscosity was reduced by 20% (PreE+) and 37% (PosE+) when enzyme was added (starter birds) in the diets as compared to E-. The improved broiler performance with enzyme addition can also be partly attributed to higher AME with enzyme supplementation. Steenfeldt et al. (1998b) also found improvement in AME and apparent digestibility of protein and fat with enzyme supplementation.

The interaction effect of enzyme and temperature on FCR (Figure 4.6) and AME (Figure 4.7) in starter birds is not clear in this study. Although positive effects of high conditioning temperature on broiler performance (e.g., FCR observed here) have been established, the results are not always consistent, particularly for estimates of digestibility of diets such as AME. Svihus et al. (2004b) demonstrated that conditioning (75°C) improved AME and performance of broilers compared to the birds fed a mash diet. Nir et al. (1995) found similar results with feed conditioned at 85°C. However, Silversides and Bedford (1999) found better performance at 80°C in comparison to the feed conditioned at 95°C. The fact that application of high conditioning temperature appears to improve the nutritive value of broiler diets may be explained by an increase in gelatinisation of starch, degradation of heat labile antinutritive factors and destruction of cell walls, improving the availability of nutrients (Pickford, 1992). The improvement of FCR with higher temperature in this study may also be accounted for

improvement in pellet durability with higher temperature (Abdollahi et al., 2010b; Lundblad et al., 2011). However, in these studies, there was no effect of conditioning temperature (60 vs 75 vs 90°C) on AME and FCR.

Despite the fact that, a moderate increase in conditioning temperature is beneficial, use of excessive heat during conditioning can deactivate enzymes and vitamins and reduces nutrient availability (Pickford, 1992; Silversides and Bedford, 1999). Additionally, there is a general assumption that a high conditioning temperature increases digesta viscosity by releasing previously encapsulated NSP and increasing the solubilisation of polysaccharides (Westerlund et al., 1989; Cowieson et al., 2005). Conventional thought would indicate that this should have been increased with higher temperatures. There were main effects of reduced digesta viscosity with EAM and that is consistent. Similarly, with main effect of CT, digesta viscosity decreased with higher temperature, when it was expected to have increased. The digesta viscosity of diet without supplemental enzyme conditioned at low temperature (65°C) was higher than the post-pellet enzyme addition. In both of the starter and grower pellets, there was significantly higher retention of the enzyme activity when enzyme was supplemented post-pelleting, and there was slightly lower levels with 95°C as compared to 65°C.

The impact on gut segment size can be explained as, without enzyme, the viscosity of digesta is increased and slows the passage of digesta contents; in response GIT secretion and motility is increased. This might have led to increased tract size. This result is consistent with the studies of Wu et al. (2004). In contrast, in some studies there was no effect of xylanase supplementation on digestive tract measurement (Engberg et al., 2004; Esmaelipour et al., 2011). The effect of vacuum coating on digestive tract length can partly be explained by the protection of enzyme from upper gut due to coating and availability of enzyme for reduction of NSP.

4.6 Conclusion

In can be concluded that xylanase supplementation and high temperature (up to 95°C) have positive impacts on broiler performance. Post-pellet heat-sensitive enzyme application can be used to improve enzyme efficacy. Vacuum coating seems to protect the enzyme activity but any effect on performance was not observed. It is recommended that studies be focused on the use of vacuum coating with different vacuum conditions, ingredients and pellet processing parameters. Future studies should also look at reduced levels of bioactives, i.e., below the recommendations of the suppliers, to further test the impact of enzyme application methods, conditioning and pelleting conditions, and efficacy of vacuum coating. If recommendations account for losses during conditioning and pelleting, this will reduce the ability to show improvement with vacuum coating, and potentially reduce enzyme costs to help balance vacuum coating costs. It is also recommended for use of bioactives that can be applied and measured accurately.

5.0 EFFICACY OF POST-PELLET ENZYME APPLICATION USING VACUUM COATING ON CHANGES IN ENZYME ACTIVITY AND PELLET QUALITY DURING STORAGE OF PELLETED BROILER DIETS

5.1 Abstract

An experiment was conducted to evaluate the effect of post-pellet applications of enzyme using vacuum coating (VC) when pellets were stored under controlled (37°C) temperatures for up to 120 d. There were two enzyme addition methods (EAM; pre-pellet addition of enzyme, PreE+, and post-pellet addition of enzyme, PosE+), two coating methods (CM; without vacuum, VC- and with vacuum, VC+) and five storage times (ST; 0, 15, 30, 60 and 120 d). Enzyme activity, feed extract viscosity and pellet durability index (PDI) were measured (n=2) at each ST. Analysis was carried out using a 2×2×5 factorial arrangement completely randomised design with two EAM, two CM and five ST. There was a 3-way interaction ($P<0.01$) of EAM, CM and ST on enzyme activity, which indicated no effect of CM for PreE+ regardless of ST. However, with PosE+, enzyme activity was retained at significantly higher levels with VC+ after 30d of ST. Diets with PosE+ had significantly ($P<0.01$) lower extract viscosity compared to PreE+. Extract viscosity was also affected ($P<0.01$) by ST. Extract viscosity at 30 d was significantly lower than at 15 d, but not different from 0 d. There was no difference of extract viscosity at 60 and 120 d; however, both were lower than at 0 and 30 d. The PDI of the diets was reduced with storage. In conclusion, vacuum coating resulted in significantly longer retention of xylanase activity during prolonged storage of feed when enzymes were applied post pelleting.

5.2 Introduction

There are few reports on the stability of enzymes in feed during storage. Although storage time of feed should be minimized, this may not be possible when feed is exported and required to

have a longer shelf-life under varying conditions. Feed additives like xylanse are susceptible to storage conditions and this is minimized by the use of stabilizers (El-Sherbiny and El-Chaghaby, 2012). Liquid enzymes are more susceptible to storage conditions than their granular counterparts because there is sufficient water to remain active (Steen, 2001). When liquid enzymes are applied to pellets, most remains on the outer surface of the pellets and may get lost in the fines during storage (Engelen and van der Poel, 1999). Therefore, the physical integrity of the pellets also is important for protection of activity of feed additives if feeds are stored for extended periods. Enzymes on the surface of feed would also be in contact with air and damaged by oxidation.

Despite vacuum coating being used primarily to increase the inclusion of fat in extrudates and pellets, this technology may offer an opportunity to encapsulate feed additives in pellets and prevent loss of activity during storage. Further, it may also reduce losses associated with fines as vacuum coating draws the bioactives deeper into the pellets and extrudates. The objective of this study is to evaluate if vacuum coating of xylanase could provide protection of enzyme when pellets are stored for up to four months. In addition, the effect of storage on extract viscosity and pellet durability index (PDI) also was evaluated.

5.3 Materials and Methods

5.3.1 Sample storage and analysis

The 12 grower/finisher diets described in chapter 4.0 were sampled and stored for up to 4 months in a commercial egg incubator (Petersime, Model 5, Gettysburg, OH, USA)) maintained at 37°C. However, only the diets that were treated with enzyme either pre- or post-pelleting are included in this report. Furthermore, the data for conditioning temperature was combined as initial evaluations showed no effect on the parameters measured during storage. The respective

diet samples were measured at 0, 15, 30, 60 and 120 d for enzyme activity, extract viscosity and PDI. All measurements were replicated for each storage time. The procedures for measurement have been previously described in Chapter 4.0.

5.3.2 Statistical analysis

Statistics were completed for a 2×2×5 completely randomised design for two enzyme addition methods (EAM; PreE+, pre-pellet enzyme addition; and PosE+, post-pellet enzyme addition), two coating methods (CT; VC-, without vacuum; and VC+, with vacuum), and five storage times (ST; 0, 15, 30, 60 and 120 d).

Analysis was carried out using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc, 1996) and mean separation was performed by Tukey's test. Significance level was set at $P \leq 0.05$ and trend set at $0.05 < P < 0.10$. Regression and correlation analysis was performed using Proc REG and Proc CORR method of SAS 9.3, respectively.

5.4 Results

The average values for enzyme activity, extract viscosity and PDI are presented in Table 5.1. There was a three-way interaction of EAM, CT and ST on enzyme activity (Figure 5.1). The interaction indicates that there was no significant difference between CM (VC-and VC+) on each storage day, when enzyme was added pre-pelleting. However, with PreE+ with either VC- or VC+ the enzyme activity was lower at 120 d compared to other days of measurement. For PosE+, a difference between CM was observed after 15 days of storage, and the difference due to CM increased with storage time. The enzyme activity of post-pellet vacuum-coated (PosE+_VC+) pellets was higher when measured on 30, 60 and 120 d of ST in comparison to PosE-_VC-. There was a significant linear response for decreasing enzyme activity during storage for both PreE+ ($R^2 = 0.59$) and PosE+ ($R^2 = 0.40$) (Figure 5.2).

Table 5.1 Effect of enzyme addition method (PreE+, pre pellet addition of enzyme or PosE+, post-pellet addition of enzyme), coating method (VC-, without vacuum application or VC+; with vacuum application), and storage time (0, 15, 30, 60, 120 days) on enzyme (xylanase) activity (n=4), diet extract viscosity (n=2) and pellet durability index (PDI) (n=2) in wheat-rye based broiler grow/finish pellets.

Treatments[1]	Level[2]	Enzyme activity (Unit/kg)[3]	Extract Viscosity (cP)[4]	PDI (%)[5]
Enzyme addition method (EAM)	PreE+	1091[b]	9.0[a]	87.1
	PosE+	2063[a]	4.5[b]	86.4
SEM[6]		*19.1*	*0.18*	*0.48*
Coating methods (CM)	VC-	1464[b]	6.9	86.4
	VC+	1689[a]	6.5	87.1
SEM		*19.1*	*0.18*	*0.48*
Storage time (ST; d)	0	1775[a]	7.9[a]	90.9[a]
	15	1731[ab]	6.6[b]	90.4[a]
	30	1623[bc]	7.7[a]	90.3[a]
	60	1551[c]	6.1[bc]	85.2[b]
	120	1203[d]	5.3[c]	76.9[c]
SEM		*30.3*	*0.28*	*0.79*
P-values of main effects and interactions				
EAM		*<0.01*	*<0.01*	*NS*
CM		*<0.01*	*NS*	*NS*
ST		*<0.01*	*<0.01*	*<0.01*
*EAM*CM*		*<0.01*	*NS*	*NS*
*EAM*ST*		*NS*[7]	*NS*	*NS*
*CM*ST*		*<0.01*	*NS*	*NS*
*EAM*CM*ST*		*<0.01*	*NS*	*NS*

[1,2] EAM; enzyme addition method, PreE+; pre pellet addition of enzyme, PosE+, post-pellet addition of enzyme, CM; coating method; VC-; without vacuum application ,VC+; with vacuum application, ST, Storage time (0, 15, 30, 60, 120 days)

[3] Enzyme activity is measured as Unit/kg as described in materials and methods of chapter 4.0

[4] Viscosity is measured in centipoise (1 cP=0.01 g/cm/s)

[5] Pellet durability index

[6] Standard error of mean

[7] Not significant

[a-d] Means within columns for each item with no common superscripts are significantly different ($P \leq 0.05$)

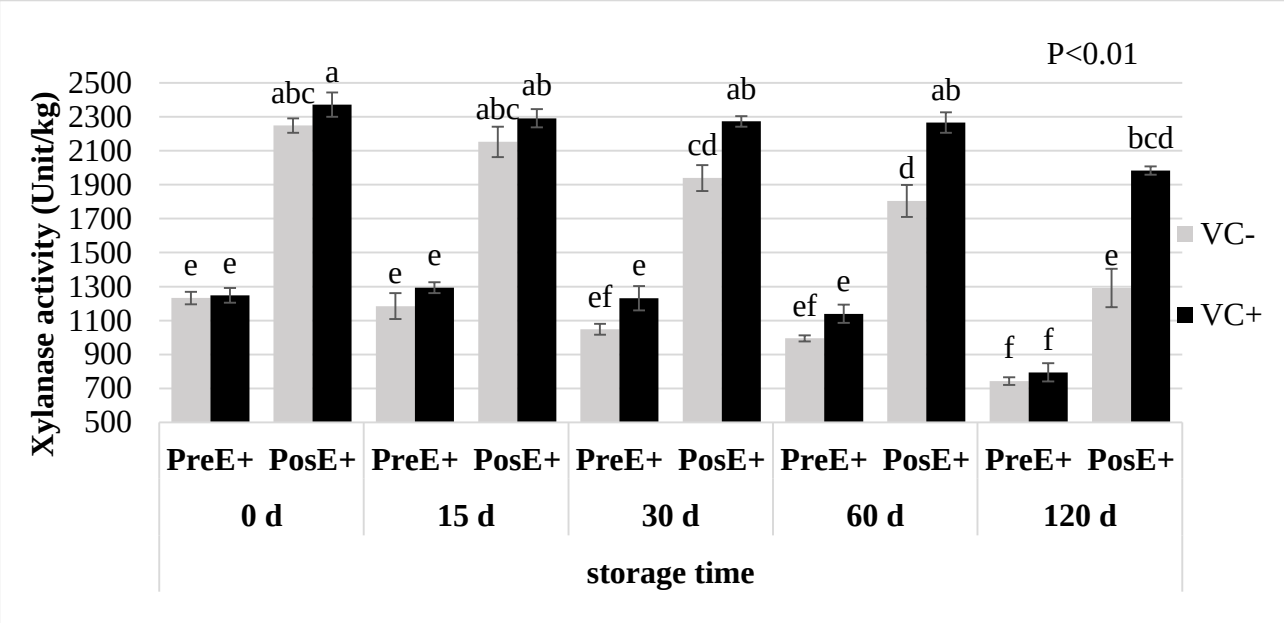

Figure 5.1 Three way interaction of EAM, ST and CM on enzyme activity (n=8) of wheat-rye based broiler grow/finish diet. (EAM; enzyme addition method, PreE+; pre pellet addition of enzyme, PosE+, post-pellet addition of enzyme, CM; coating method; VC-; without vacuum application ,VC+; with vacuum application, ST, Storage time (0, 15, 30, 60, 120 d))

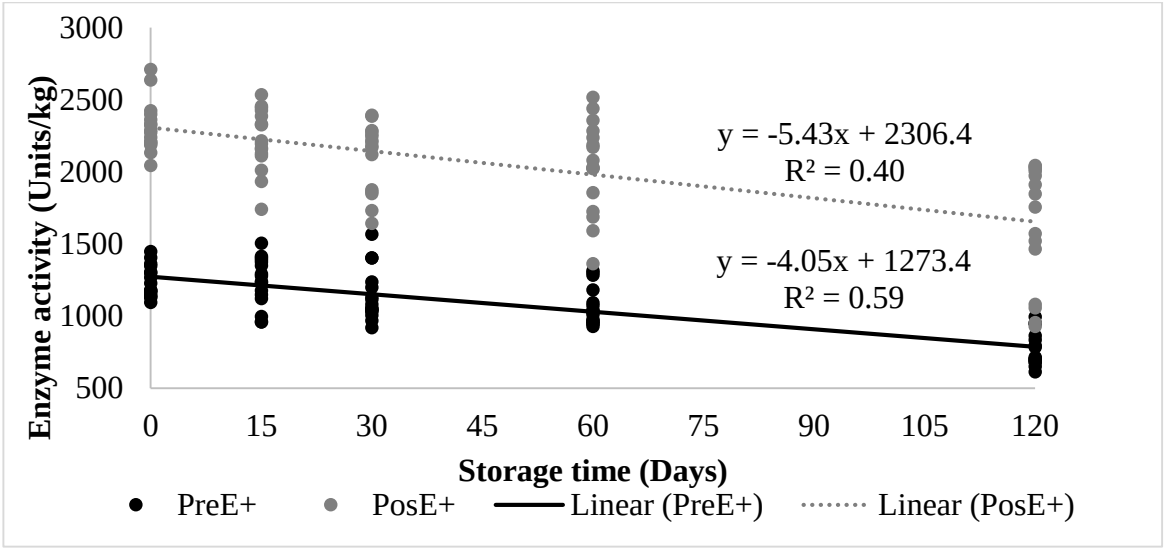

Figure 5.2 Linear regression analysis to evaluate the effect of storage time on xylanase activity (n=48) when added pre or post pelleting in wheat-rye based broiler grow/finish pellets (PreE+; Pre-pellet addition of enzyme, PosE+; Post-pellet addition of enzyme)

There was a significant effect of EAM (P<0.01) and ST (P<0.01) on extract viscosity of the feed. Post-pellet addition of enzyme reduced the extract viscosity of the feed. Extract viscosity at 30 d was higher than at 15 d but not different from 0 d. There were no differences in extract viscosity at 60 and 120 d; however, both were lower than at 0 and 30 d. Similar to enzyme activity, extract viscosity also decreases linearly for both PreE+ (R^2 = 0.20) and PosE+ (R^2 = 0.57) as storage time increases (Figure 5.3).

There was no effect of EAM on PDI. However, ST affected PDI. There was no difference in measurement of PDI at 0, 15 and 30 day. Pellet durability index was reduced by 5.6% on 60 d when compared to 30 d and further reduced by 9.7% on 120 d when compared to 60 d. The decrease in the PDI along with the storage time was linear (R^2 = 0.71; Figure 5.4)

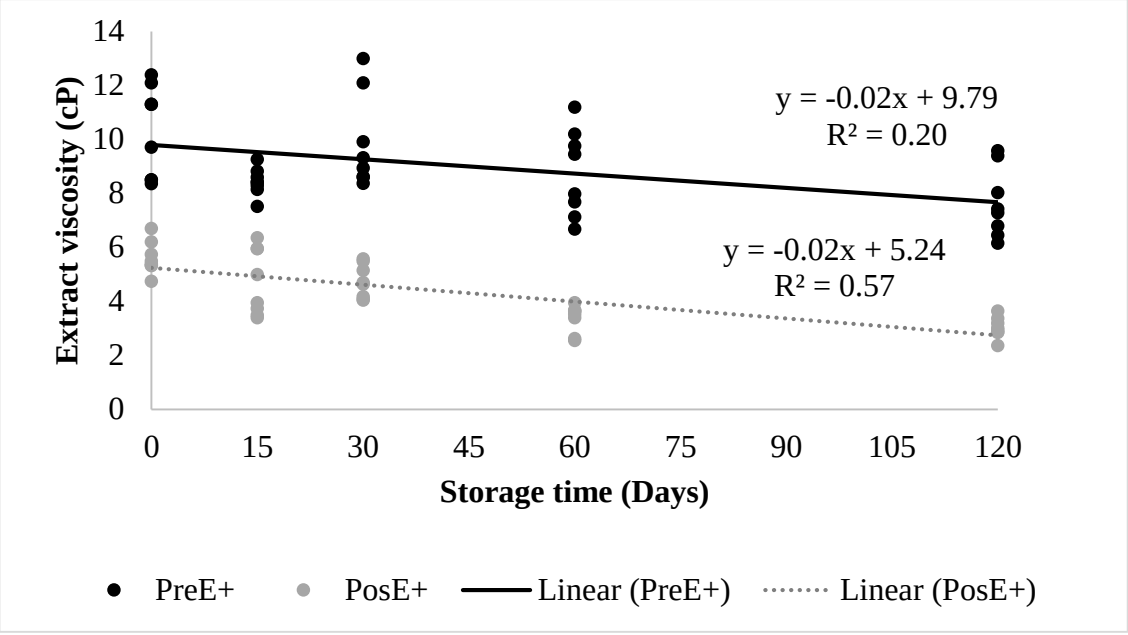

Figure 5.3 Linear regression analysis to evaluate the effect of storage time on extract viscosity (n=24) when xylanase was added pre or post pelleting in wheat-rye based broiler grow/finish pellets (PreE+; Pre-pellet addition of enzyme, PosE+; Post-pellet addition of enzyme)

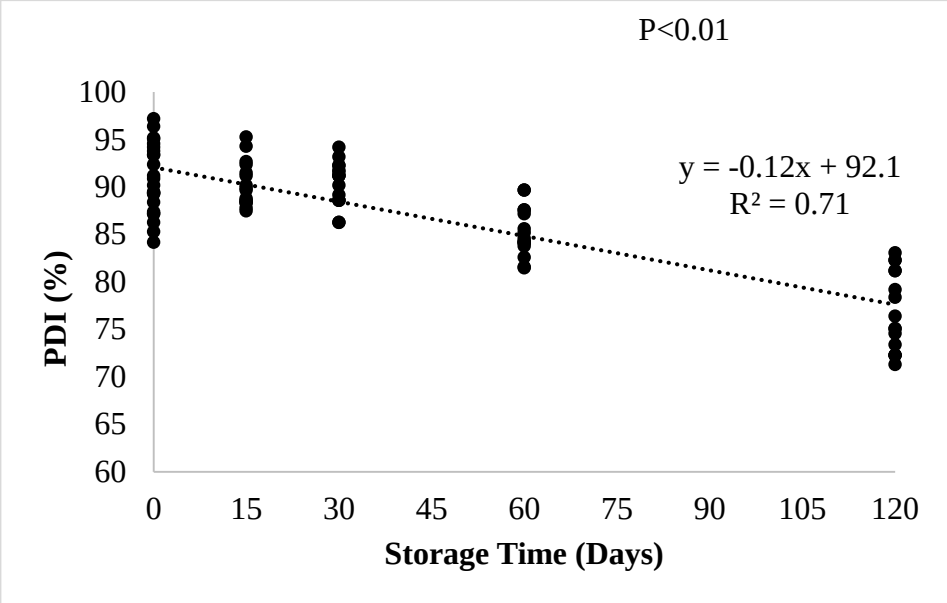

Figure 5.4 Linear regression analysis to evaluate the effect of storage time on pellet durability index (PDI, n=24) of wheat-rye based broiler grow/finish pellets

The Pearson correlation coefficients for enzyme activity, extract viscosity and PDI of the pellets are presented in Table 5.2. It was found that viscosity and enzyme activity were negatively correlated. Similarly, PDI had a positive correlation with enzyme activity and extract viscosity.

Table 5.2 Pearson correlation coefficient (r) of enzyme activity (n=160), extract viscosity (n=80) and PDI (Pellet durability index; n=88), when stored up to 120 days and parameters measured on 0, 15, 30, 60 and 120d.

	Enzyme activity	Extract viscosity	PDI
Enzyme activity	1.00	-	-
Viscosity	-0.66**	1.00	-
PDI	0.22*	0.35**	1.00

* P<0.05, **P<0.01

5.5 Discussion

Vacuum coating technology is an important means of increasing fat inclusion in extrudates and pellets and was evaluated in the present study for efficacy of applying liquid enzymes in pelleted diets. Certain liquid additives, such as xylanase are susceptible to loss of bioactivity when feed is stored at a high temperature (>22°C; Steen, 2001). However, once liquid enzymes have been applied to the feed, they become inactive due to reduction of moisture by dilution with the diet (Steen, 2001). This may not be the case when the liquid enzyme is coated post-pelleting and subjected to loss in the fines. The principle of vacuum coating is to have the liquid applied taken up into the core of the pellet. In the case of liquid enzyme addition, this may help reduce losses of enzyme in fines generated during storage of feed. Similar results were observed by Engelen and van der Poel (1999), who reported the activity of xylanase was higher in fines than in pellets when enzyme was sprayed on the feed in a batch mixer. This is supported by the decreasing PDI measured in the current study with increasing storage. Pellet durability was reduced significantly at 60 d and further reduced at 120 d. This shows that as the ST increased, pellets started to degrade, contributing to a higher level of fines and ultimately loss of enzyme in the fines. This is more prevalent in case of PosE+_VC-. In contrast, when feed was vacuum-coated, the enzyme was not lost in the fines despite the loss in durability. This might be the reason for the positive correlation of enzyme activity and PDI. Enzyme activity in fines was not measured in the present study, but it is recommended to measure in future studies

The general assumption is that, as the activity of xylanase decreases, the extract viscosity of feed should be higher (explained in Chapter 4.0). This was consistent when viscosity was compared between PreE+ and PosE+, which showed higher extract viscosity in PreE+ and which was associated with lower enzyme activity. The extract viscosity at 60 and 120 d of storage was

reduced despite a decrease in enzyme activity. This could be due to a decrease in the level of arabinoxylan with storage, although this was not measured. Similar results was observed when Fuente et al. (1998) stored barley for up to 32 week before including it in poultry diets. In this case both endogenous β-glucanase activity and viscosity was reduced and measurements were positively correlated (r=0.86). The extract viscosity and nutritive value of grains vary during storage, and this is a function of grain source, processing (e.g. grinding and/or pelleting) and storage conditions (George and McCracken, 2003). The majority of studies have been focused on storage of grain rather than a complete feed. The studies related to effect of storage on nutritive value of complete pelleted feeds, physical properties of pellets and the stability of additives are rare, which needs attention.

5.6 Conclusion

In addition to the use of vacuum coating technology as a tool to increase fat inclusion and protection of bioactives, it can be used to improve the shelf-life of bioactives. Vacuum coating proved to encapsulate the liquid feed additives and reduce the losses in fines when feed was stored for more than two months. Storage conditions and the type of feed additives might affect the retention of bioactivity of the additives. Further work is required to understand the interactions between the use of vacuum coating technology (e.g. different settings such as vacuum pressure, spray pressure, mixing time etc.) and sources of bioactives when held under different storage conditions.

6.0 OVERALL DISCUSSION AND FUTURE DIRECTIONS

A better understanding of the interactions between ingredients and processing conditions offers several opportunities to add value to animal feed. Use of low energy-high protein co-products (e.g., oilseed meals or biofuel DDGS) is replacing cereal grains in broiler diets and energy needs to be balanced by the addition of fats and oils. In the case of biofuels, this has been further exacerbated by removal of the oil in corn for biodiesel production, thereby further reducing energy in distillers dried grains. However, the requirement for higher fat inclusion in diets is limited due to negative effects on pellet durability. Hence, alternative methods of post-pellet fat inclusion in pelleted diets, such as vacuum coating, need to be evaluated.

Vacuum coating was found to be an option for inclusion of a high-level of fat in broiler pellets. One important limitation of the use of vacuum coating of broiler pellets would be high pellet density/low porosity. Pellets are produced by hydrothermal conditioning of the mash. The use of high temperature has become common practice in modern feed manufacturing as a means of improving pellet quality and digestibility and for sterilization of the feed. However, there may be negative effects of higher heat, including destruction of heat-sensitive compounds, such as enzymes, and an increase in soluble NSP resulting in an increase of diet viscosity. To what extent these considerations lead to over formulation of feed additives and negative effects on animal performance needs to be better understood. Therefore, research was conducted to see if grain type and particle size had any effect on pellet porosity and post-pellet oil absorption by vacuum coating. The use of vacuum coating of heat-sensitive bioactives (enzyme) also was evaluated.

Vacuum coating was found to be effective in increasing the fat level in pellets. There are only a few studies that demonstrate vacuum coating could increase the fat level in broiler

pelleted feed (Strauch, 2002; Borequez and Perez, 2007). Additionally, important information about the effect on particle size of different grain sources when ground through the same hammer mill screen was found. In the case of barley, the particle size was always higher than for wheat and corn regardless of screen size. Further, larger particle size was found to increase porosity and oil absorption, as was reported by Strauch (2002). However, there was an exemption with coarse ground barley having lower oil absorption (Table 3.1). This may be a function of the source of barley or chemical and physical factors (e.g. fiber from the hull) affecting porosity and pore size and thereby negatively impacting oil absorption or loss of oil through leakage. It would be interesting to see how oil absorption and porosity behave with complete feed pellets and their interaction with alternating processing conditions of pelleting such as temperature, moisture and die specification.

Until now, there are no studies reporting the use of vacuum coating to protect heat-sensitive feed additives during pelleting and storage of pellets. This study suggests no effect of vacuum coating of xylanase on broiler performance. However, post-pellet vacuum coating of enzyme indicates it can be used to achieve higher enzyme retention in comparison to post-pellet coating without vacuum coating. Further, vacuum coating was able to encapsulate enzyme and protect them during storage. This could lead to further opportunities to compare impact of vacuum coating for other bioactives such as probiotics or vaccines.

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8.0 APPENDICES

Appendix 1 Poultry Science Abstract (2014); Understanding the importance of particle size to improve oil inclusion in pellets with or without vacuum coating

Poultry Science Conference July 14-17, 2014, Corpus Christi, Texas, USA

Understanding the importance of particle size to improve oil inclusion in pellets with or without vacuum coating

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Vacuum coating is not usually associated with pelleted broiler feed. However, if vacuum coating can economically improve broiler performance, then pellet porosity and liquid uptake will become more important. An experiment was conducted to investigate the effect of particle size on post-pellet fat absorption with (V+) and without (V−) vacuum coating. Three different grains; wheat, barley and corn were ground with hammer mill using 2 different screens (3.17 and 6.35 mm). Two ground and a whole grain (i.e., 3 particle sizes) for each of the 3 grains were pelleted using a 4.7-mm die. Following cooling, samples of intact pellets were prepared for additions of canola oil (15% by weight) that was deemed to be in excess of pellet surface area and porosity. Statistical analysis was carried out separately for 3 grain types; duplicate measurements of oil uptake were taken and analyzed based on completely randomized design for particle size (n = 3) and coating method (V+ and V−) for each of the grain. With coarse grinding, particle size was highest for barley (1,896 ± 54.5 µm), then wheat (1,289 ± 46.5 µm) and corn (1,057 ± 59.11 µm). The particle sizes of finely ground grains were 1153 ± 81.0 µm, 767 ± 36.0 µm and 732 ± 21.0 µm for barley, wheat and corn, respectively. Overall, there was a 1.4 ± 0.36% higher oil uptake when vacuum (V+) was applied during oil addition and mixing in all of the pellets. There were significant differences in amount of canola oil absorbed in all grain sources with different particle size ($P < 0.05$). The highest oil absorption (11.6%, V+; 9.7%, V−) was found in pellets produced from barley that was finely ground. However, for wheat and corn, pellets produced from coarse particle size absorbed more oil than pellets from fine particle size, both with and without vacuum coating. The pellets formed from whole grain absorbed the least amount of oil ($P < 0.05$) in all grain sources, both with (V+; 8.2, 8.8 and 4.2%) or without (V−; 6.9, 7.4 and 3.2%) vacuum, respectively for wheat, barley and corn. In conclusion, particle size of the grain can be manipulated to get higher post-pellet fat absorption, using both coating methods.

Appendix 2 Animal Feed Science and Technology Abstract (2015) Vacuum coating of pelleted feed for broilers: Opportunities and challenges

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Vacuum coating of pelleted feed for broilers: Opportunities and challenges

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Post-pellet application of fats and protection of heat-sensitive bioactives can be achieved through vacuum coating. This review highlights the use of vacuum coating for delivery of liquid fat or bioactives (i.e., enzymes, vitamins, probiotics, etc.) in pelleted broiler diets. Pre-pellet delivery of fats substantially reduces pellet durability and results in losses in bird performance and customer satisfaction. Vacuum coating can also improve storage life and bioavailability of bioactives sensitive to digestion in upper part of gastrointestinal tract. As for encapsulation, vacuum coating may also offer a means of improving delivery and safe handling of offensive compounds. This technology requires highly durable and porous pellets for effective application. The challenges of this technology are the need to better understand how to improve pellet porosity and increase liquid inclusion while maintaining pellet durability; bearing in mind the increased handling of pellets when they are vacuum-coated. The discussion also includes methods for measuring and manipulating the porosity of the pellets to achieve optimum pellet durability and liquid application.

Appendix 3 Poultry Science Abstract (2015); Evaluation of post-pellet vacuum coating on protection of xylanase and effect on broiler performance when fed wheat-rye-based diets

Poultry Science Conference July 27-30, 2015, Louisville, Kentucky, USA

Evaluation of post-pellet vacuum coating on protection of xylanase and effect on broiler performance when fed wheat-rye-based diets

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An experiment was conducted to evaluate the effect of vacuum coating (VC) on post-pellet application of xylanase enzyme and its impact on broiler performance when fed wheat-rye-based diets. Twelve diets were produced based on three enzyme addition methods (EAM; without enzyme (E-), enzyme added pre-pelleting (PreE+) and post-pellet enzyme addition (PosE+)), two conditioning temperatures (CT; 65 and 95 ^{O}C) and two coating methods (CM; without (VC-) and with (VC+; 0.3 bar, 1 min mixing) vacuum application). The diets were fed to 360 Ross 308 male broilers in two phases (starter; 10-21d (5 birds×6 cages/diet) and grower/finisher; 21-35d (5 birds×3 cages/diet)) to determine performance and digestibility. Enzyme activity, extract viscosity and pellet durability index (PDI) of pellets were determined. A completely randomised block design based on 3 EAM x 2 CT x 2 CM was used. There was a significant (P=0.03) 3-way interaction on 21d body weight, while only PreE+ and PosE+ improved (P<0.01) average weight at 35d. There were no significant treatment effects on feed intake of starter; however, 95 ^{O}C CT reduced (P<0.05) feed intake of grow/finish diets. Feed efficiency was significantly (P<0.05) improved by enzyme addition (PreE+ and PosE+) and 95 ^{O}C CT. There was a 3-way interaction (P=0.04) on digesta viscosity (cP) for birds sampled at 21 d (starter diets); whereas only EAM (P<0.05) was significant at 35d. There was also a 3-way interaction on pellet extract viscosity of starter (P=0.05) and grow/finish (P=0.07) diets. High temperature significantly (P<0.01) improved PDI. Enzyme activity of post-pellet enzyme coated diets was significantly (P=0.04) improved by VC+. Enzyme addition (PreE+ and PosE+) and high CT increased (P<0.05) AME of both starter and grower diets. Vacuum coating significantly (P<0.05) reduced the relative length of small intestine of broilers at 21d but not at 35d. In conclusion, VC may offer a means of delivery of heat-sensitive bioactives in broiler.