Enhancing the feed value of red grape pomace for broiler chickens using polyethylene glycol and fibrolytic enzymes

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DECLARATION

I declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Agriculture in Animal Science has not been previously submitted to any other University or institution and that it is my own original work. Material and information from other sources are fully recognized and acknowledged.

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GENERAL ABSTRACT

The use of non-conventional feedstuffs has the potential to sustainably intensify poultry production in resource-poor communities of South Africa. Red grape pomace (GP) is a feed resource that is rich in beneficial bioactive compounds with nutraceutical properties, which have useful application in poultry nutrition. However, its utility as a feed ingredient for poultry is constrained by the presence of high levels of fibre and tannins. This study was designed to evaluate and enhance red grape pomace as an ingredient in broiler chicken diets so as to contribute to food security and environmental stewardship. The objective of Experiment 1 was to identify an optimal inclusion level of GP in Cobb 500 broiler chicken diets based on growth performance measurements. Four hundred, two-week old Cobb 500 broiler chickens (279.2 ± 18.87 g) were reared using commercial grower and finisher diets to evaluate their physiological and meat quality traits in response to incremental levels of GP. For four weeks, broilers were fed five isonitrogenous and isoenergetic experimental diets containing graded levels of GP as follows: GP0 = commercial chicken diet without GP; GP25 = commercial chicken diet containing 2.5% GP; GP45 = commercial chicken diet containing 4.5% GP; GP55 = commercial chicken diet containing 5.5% GP; and GP75 = commercial chicken diet containing 7.5% GP. The five experimental diets were randomly allocated to 40 pens resulting in eight replicates per dietary treatments, with each pen carrying 10 chickens. Level of GP inclusion quadratically influenced FCR but neither linear nor quadratic effects were observed for haematology, serum biochemistry and carcass characteristics. Linear trends were observed for breast meat pH, redness and hue angle. The grape pomace containing diets had the least average weekly feed intake (AWFI) (g/bird) when compared to the commercial broiler diet. The dietary treatments did not differ in terms of carcass characteristics and internal organs of broiler chickens. The diet, GP75 promoted the highest (0.75) redness of the meat meanwhile, GP0 had the least (0.49). The hue angle was observed

to decrease as the inclusion level of GP increased with GP0 having the highest (1.54) and GP75 had the least value (1.52). However, there were no dietary effects on meat pH, meat temperature and chroma of the meat. It was established that GP can be incorporated in commercial broiler diets up to 7.5% without compromising the birds growth performance, health and meat quality. The amount of GP that can be incorporated in broiler diets is limited by antinutritional components such as fibre and condensed tannins. The fibre in GP may negatively affect digestion and absorption of nutrients while condensed tannins can bind and reduce availability of nutrients such as proteins and carbohydrates. Phenolic compounds of lower molecular weight may also get absorbed through the digestive tract and cause toxicity.

Experiment 2 was designed to evaluate strategies that would improve the intake of GP by broiler chickens by ameliorating the negative effects of fibre and condensed tannins. This was tested by including GP in commercial broiler diets at a level (10%) greater than the optimum level identified in Experiment 1 and assessing whether prior treatments of GP with polyethylene glycol and fibrolytic enzyme treatments would improve physiological and meat quality parameters of broiler chickens. The treatment of GP with polyethylene glycol before incorporation into commercial broiler diets inactivated condensed tannins while treatment with the enzyme, Viscozyme® was designed to improve fibre digestion. For four weeks, broilers were fed five isonitrogenous and isoenergetic dietary treatments formulated as follows: Commercial chicken diet without red grape pomace (CON); Commercial chicken diet containing 10% red grape pomace (GP); Commercial chicken diet containing 10% red grape pomace pre-treated with polyethylene glycol (5% w/w) (PEG); Commercial chicken diet containing 10% red grape pomace pre-treated with Viscozyme® - L (0.1% w/w) (ENZ); and Commercial chicken diet containing 10% GP pre-treated with both polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w) (PENZ). There were no (P >0.05) week × diet interaction effects on average weekly feed intake, average weight gain and FCR. The

slaughter weights of CON, PEG, ENZ and PENZ chickens did not differ (P >0.05). However, GP diet promoted the least slaughter weight (1468.4 g) in chickens. Broiler chickens on CON (1276.5 g) and PEG (1243.6 g) diets had bigger HCW, which did not differ. However, GP promoted the least (1120.6 g) HCW, which was similar (P >0.05) to that of birds fed ENZ and PENZ diets. Meanwhile, the HCW of PEG, ENZ and PENZ chickens did not differ (P >0.05). Broilers on the CON (1227.4 g) and PEG (1210.0 g) diets had higher CCW compared to GP, ENZ and PENZ fed chickens, whose CCW did not differ. Diets significantly affected the WHC of breast meat with PENZ promoting the highest WHC (8.316 %) and PEG promoting the least (5.223 %). The dressing percentage, meat cooking loss, meat shear force (meat tenderness) and meat drip loss were not affected (P >0.05) by the experimental diets. There were no dietary effects on size of most internal organs except for duodenum, ileum, jejunum and ceca. It was concluded that the inclusion of 10% GP treated with PEG resulted in chickens with similar HCW as those on the conventional commercial diet. The treated GP had similar weight gain as commercial broiler diet suggesting that the antinutritional effects of tannins and fibre were successfully ameliorated. As such, prior treatments of GP to reduce the antinutritional effects of fibre and condensed tannins improves broiler performance by boosting feed utilization efficiency while providing health benefits to consumers of broiler meat.

Keywords: Cobb 500 broilers, Grape pomace, Meat quality, Polyethylene glycol, Viscozyme® - L,

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DEDICATION

I dedicate this thesis to my parents, Mr and Mrs Vithi, and my little sister, Buqaqawuli, who have been morally supportive throughout my study.

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PEER-REVIEW ARTICLES FROM THIS THESIS

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphate
ALT	Alanine aminotransferase
AWFI	Average weekly feed intake
AWG	Average weekly gain
CCW	Cold carcass weight
FCR	Feed conversion ratio
FI	Feed intake
GP	Grape Pomace
HCW	Hot carcass weight
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
PEG	Polyethylene glycol
WHC	Water Holding Capacity

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

According to the South African Department of Trade and Industry (2017), poultry production is the leading agricultural sector, contributing more than 16% of the sector's share of the gross domestic product. It plays a pivotal role in creating job opportunities, directly and indirectly, throughout its value chain and related industries (DTI, 2017). The industry supports many large and small-scale enterprises and provides a strong platform for rural development, as well as the state food security programme, as it is the main supplier of animal protein. It has evolved, over more than hundred years, from backyard or household farming into a complex and highly integrated industry. The increasing human population as well as greater health concerns around red meat consumption, have led to an increased demand for poultry meat worldwide. Indeed, Delport *et al.* (2017) mentioned that white meat has the highest consumption rate per capita, particularly in South Africa. This high demand and consumption is attributed to the fact that white meat is relatively inexpensive and affordable compared to beef, chevon, mutton and pork. It is increasingly difficult for farmers to meet the high demand of white meat due to uncontrollable diseases, high mortality rate, droughts, cost of power and production, poor infrastructure, high feed and drug costs, and lack of technical expertise (Bounds & Zinyemba, 2018). The high cost of feeding has emerged as a major constraint to poultry production, leading to greater efforts to explore alternative feed ingredients for least-cost and effective poultry production (Wickramasuriya, 2015). The biggest challenge is the growing competition between people and intensively reared poultry for food because maize and soybean are major direct sources of feed for birds and food for human. This competition for food has not only affected the poultry farmers but it

has also resulted in crop farmers needing to increase their production in order to meet the demand for both livestock and people.

Grape (*Vitis vinifera L.*) is said to be one of the largest grown fruit crops in the world, with an approximate annual production of 61 million metric tons (Dorri *et al.*, 2012). According to the South African Table Grape Industry (SATGI), South Africa is the northern hemisphere's oldest and most reliable supplier of table grapes. More than 80% of grape production in South Africa occurs in the Western Cape region. Other production areas include the Northern Cape, Eastern Cape, Limpopo, Free State and Mpumalanga provinces. According to the South African Table Grape Industry (2010), 95 775 hectares of vines producing wine grapes are under cultivation in South Africa over an area some 800 km in length. In 2010, table and dry grapes contributed 31% (23 532 ha) of the total area planted to deciduous fruits (75 025 ha). Cuccia (2015) defined grape pomace is the main solid organic waste (seeds and skin) from winery industries; resulting from the pressing and/or fermentation processes. It is generated in large amounts in many parts of the world (Abarghuei *et al.*, 2010; Christ & Burrit, 2013) and studies have shown that pomace represent about 20 – 30% of the original grape weight (Dwyer *et al.*, 2014). The amount of grape pomace generated from winemaking is dependent on the grape cultivar, the pressing process, and the fermentation steps.

1.2 Problem statement

Diet is a key factor in animal production, since it does not only affect the health and productivity of farm animals, but also the cost of livestock products (Alders *et al.*, 2009). Grape pomace is a potential feed resource for poultry because it is relatively inexpensive, has no direct food value for humans and is readily available. The use of GP will help overcome problems of feed shortage and high production cost and at the same time ensuring the preservation of animal health, production yield and product quality. As grain production

remains insufficient to meet human and animal feeding, the alternative is to employ feed ingredients, which do not have direct food value for human.

Prophylactic antibiotics are used regularly in animal feed to prevent subclinical infections, increase feed utilization efficiency and growth rate. The use of antibiotics in animal feed may cause increased resistance to antibiotics allowing resistant bacteria to proliferate in the animal and possibly in humans (Dale, 1992). In addition, the presence of antibiotic residues in poultry products is a major health concern for consumers of these products (Menten, 2001). For this reason, there is a trend to reduce the use of antibiotics in animal feed. Grape pomace possesses a substantial amount of polyphenolic compounds that have antimicrobial activity and thus has the potential to be used to control the growth of pathogenic microorganisms *in vivo* and thus enhance animal performance.

Large amounts of grape pomace are produced during a short period of harvesting, which increases their concentration per unit area. The currently used traditional pomace disposal methods of incineration or discarding in landfills are detrimental to the environment. The phenolic compounds present in grape pomace reduces the pH of the pomace and increases its resistance to biological degradation. Other environmental problems includes surface and ground water pollution, foul odour, flies and pests attraction that may spread diseases and oxygen depletion in soil and ground waters by tannins and other compounds (Christ & Burrit, 2013; Dwyer *et al.*, 2014). It is, therefore, important to find alternative uses for GP to reduce its negative environmental effect while improving broiler productivity at less cost.

1.3 Justification

It is estimated that only 3% of grape pomace produced is reused for animal feed (Dwyer *et al.*, 2014; Brenes *et al.*, 2016). Polyphenols contained in the GP have been shown to reduce toxicity caused by free radicals and prevent oxidative damage of biological macromolecules. Moreover, they contribute significantly to defence of animal organisms by increasing the levels of endogenous antioxidant molecules and enzymes such as glutathione (GSH) and catalase (CAT) thus enhancing immune response. Grape pomace, therefore, constitutes a cheap source for antioxidant polyphenols that can be used as dietary supplements (Alonso *et al.*, 2002). Grape pomace is a rich source of flavonoids including monomeric phenolic compounds, such as catechins, epicatechin, dimeric, and tetrameric procyanidins as well as proteins, carbohydrates, fats, and minerals (Pop *et al.*, 2015). Studies have shown that flavonoids have the ability to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz & Toledo, 2004). Indeed, antioxidant activity is the most notable bioactivity of phenolic compounds from GP (Xia *et al.*, 2010; Georgiev *et al.*, 2014). The inclusion of GP in chicken diets rich in polyunsaturated fatty acids (more susceptible to oxidative processes) has been reported to delay meat lipid oxidation (Chamorro *et al.*, 2012). Other studies (Goni *et al.*, 2007; Brenes *et al.*, 2008) indicate that the intake of grape pomace increases antioxidant capacity in breast and thigh meat of broiler chickens but the addition of GP in the chicken diets does not have an impact on growth performance. Hughes *et al.* (2005) reported growth depression in chickens fed diets containing grape seed extract. According to Chamorro *et al.* (2012) GP contains high level of fibre and polymeric polyphenols as procyanidins with capacity to bind and precipitate both dietary and endogenous proteins thus the incorporation of GP at high doses in chicken diets might impair nutrient digestion and growth.

Due to the functional properties of bioactive compounds in GP, using this by-product as a feed ingredient may positively alter the metabolism and physiology of animals producing beneficial effects. The use of these bioactive compounds can provide opportunities for adding value to poultry products, reducing feed costs while decreasing the negative environmental impacts associated with disposal of grape pomace. However, grape pomace also has anti-nutritional components such as fibre and tannins, which reduce its utilization by broiler chickens. Little is known on the effect of condensed tannin-ameliorating polyethylene glycol and fibrolytic enzyme treatment of dietary red grape pomace on nutrient utilization, haemo-biochemical parameters, growth performance, and meat quality traits of broiler chickens.

1.4 Objectives

The study was designed to assess the effectiveness of condensed tannin-ameliorating polyethylene glycol and fibrolytic enzymes as strategies to enhance the feed value of red grape pomace for broiler chickens. The following specific objectives guided the study:

1. To determine the growth performance, haemo-biochemical parameters, carcass characteristics and meat quality traits of Cobb 500 broiler chickens fed diets containing incremental levels of red grape pomace.
2. To identify an optimal inclusion level of red grape pomace in Cobb 500 broiler chicken diets based on growth performance.
3. To determine the effect of polyethylene glycol and enzyme treatment of red grape pomace on growth performance, haemo-biochemical parameters, carcass characteristics and meat quality traits of Potchefstroom Cobb 500 broiler chickens.

1.5 Hypotheses

1. The first experiment explored the hypothesis that physiological parameters and meat quality traits in Cobb 500 broiler chickens respond to incremental levels of grape pomace in a non-linear fashion.
2. The second experiment tested the hypothesis that treating GP with polyethylene glycol and/or fibrolytic enzymes improves physiological parameters and meat quality traits in Cobb 500 broiler chickens.

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2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

The commercial poultry industry, which is highly organised and uses very sophisticated technology, contributes more than 17% of the South African agricultural total value(South Africa Poultry and Products Report, 2011). The broiler industry currently produces an average of 18.6 million broilers per week and has been growing steadily since 1990, when only 7.6 million broilers per week were produced (South Africa Poultry and Products Report, 2011). It is a fast developing enterprise that is characterized by intensive management, mechanization and specialization, dominated by a few large companies who are both breeders and producers (Pedersen, 2002). Productivity of birds has increased considerably in the recent years, primarily due to changes in genetic potential. The altered genetic makeup of bird is known to influence the utilization of dietary nutrients (Shafey *et al.*, 1990; Hurwitz *et al.*, 1995). The main challenge in the poultry industry are the high input (feed and drugs) costs, hence the efforts to identify alternative feed ingredients that are cheaper while acting as nutraceuticals.

Grape (*Vitis vinifera*) is one of the largest fruit crops in the world, with approximately 61 million metric tons annual production. Grape pomace (GP) is the residue left after juice extraction by pressing grapes in the wine industry. Grape pomace provides a rich source of polyphenols that have the capacity to act as powerful antioxidants. The use of GP in poultry industry is limited due to low protein availability and high phenolic content. However, grape pomace could be included in poultry diets but its inclusion rate need to be further investigation to ensure safe and beneficial utilization without compromising growth performance and health status of chickens. The presence of fibre and phenolic compounds in GP may constraint the utilization of this potential feed resource in chicken diets.

2.2 Broiler chicken farming

The South African broiler industry contributes to economy with a gross producer value of over R5 171 million per annum. Employing approximately 57 804 staff in the formal sector and main input supply industries. In 1999, about 9.8 million broilers were produced per week with per capita consumption increasing over a ten-year period from 15.5 kg to 18.5 kg. The broiler industry contributes 16.2 % to the total gross value of agricultural production. South Africa is still unable to produce adequate quantities of broiler meat to meet demand, with the shortfall being addressed through imports (DTI, 2017). Import statistics show that South Africa imported 528 506 tons of chicken meat in 2016 compared to 457 374 tons in 2015. The poultry industry faces several significant challenges that have hindered its competitiveness and growth potential. The principal challenges pertaining to the industry are rising feed costs, import penetration, rising electricity tariffs and access to reliable supply, exchange rate fluctuations and access to finance and markets.

Broiler chicken production is increasingly popular amongst the poor rural communities in South Africa. Broiler chickens serve many functions, which include the provision of meat for home consumption and income from live sales (Bett *et al.*, 2013). Portions of a human diet include chicken products, which are economically desirable protein sources (Memon *et al.*, 2009). Chicken meat and eggs are regarded as important food products for meeting the dietary needs of millions of malnourished children below the age of 5 in predominantly rural Africa (Rosegrant & Cline, 2003). In this sense, their availability and productivity in rural areas contributes to poverty reduction, through improved human health (Pica-Ciamarra & Dhawan, 2009). Poultry meat consumption especially in developing countries, when compared to the 2007-2009 base period, showed an increase of 38% in 2019 (FAO, 2010). Therefore, it is important to find ways to improve the nutrition of broiler chickens in order to meet the demand of consumers and to ensure higher profits for those farming in rural areas.

2.3 Grape pomace

Grape is a fruit widely grown and eaten around the world because of its benefits on human health. After the grapes are harvested, they are processed into wine or consumed by humans. For winemaking, grapes are crushed to extract juice containing the sugars, glucose and fructose, for fermentation (Grainger & Tattersall, 2005). Grape pomace is the fibrous material that remains after the juice has been extracted from grape berries and consists of processed skins, seeds and stems (Hang, 1988; Mazza, 1995). Grape pomace can be classified as red, white or rose pomace. The chemical components of GP include water, proteins, lipids, carbohydrates, vitamins, minerals and compounds with important biological properties such as fibre, vitamin C and phenolic compounds (tannins, phenolic acids, anthocyanins and resveratrol). The concentration of these compounds in GP depends on the type of pomace, the cultivar, growth environment, cultivation conditions (Sousa *et al.*, 2014), and processing and fermentation conditions. The GP bioactive compounds are capable of altering the metabolism and human physiology producing beneficial health effects. The exploitation of these bioactive substances can improve poultry nutrition and quality of poultry products thus contributing to the improved consumer health; reduce feed costs while ensuring good environmental stewardship. During the production of wine, grape pomace is separated from the grape juice prior to the fermentation of white wines, or after a few days of skin contact in red wines (Prescott *et al.*, 1993). In order to understand the benefits of GP as a feed ingredient, it is necessary to evaluate its impact on growth rate and physiological response when fed to animals. According to Nistor *et al.* (2014), GP is a good source of fibre and, therefore, may be used in small quantities in ruminant diets to meet the requirements of energy and nitrogen. According to Bertol *et al.* (2017), the inclusion of grape pomace in the diet of pigs to assess its effect on pork quality and oxidative stability of omega-3 enriched fat resulted in color saturation index of meat. Goni *et al.* (2007) evaluated the effect of dietary GP and vitamin E

on growth performance, nutrient digestibility and susceptibility to meat lipid oxidation in chickens. However, there is no documented literature on enhancing the feed value of red grape (*Vitis vinifera* var. Shiraz) pomace for broiler chickens using polyethylene glycol and feed enzymes, which may enable the inclusion of high levels of GP in poultry diets.

2.3.1 Nutritional composition of grape pomace

To be able to determine the alternative uses of grape pomace bioactive compounds, quantification of the bioactive compounds must be performed. Grape pomace composition varies significantly, depending on grape variety and the equipment used during wine making. In South Africa, grapes are abundant and are mainly used for wine making and human consumption. After fermenting (maceration) and pressing, grape seeds and skins remain as marc, which still contain some anthocyanins and polyphenols. Recently, attention has been on these phenolic compounds because of their health benefits, such as antioxidant activity where they act as free radical scavengers and inhibit lipoprotein oxidation. The chemical composition of grapes may vary due to extrinsic factors such as climatic conditions, viticultural practices, as well as intrinsic factors such as variety, maturity, and sanitary conditions.

According to Basalan *et al.* (2011), GP nutritional properties also differ with method of wine production, type of grape and the relative ratios of seeds, pulp, skin and stalk in the pomace. When compared to white GP, red GP had higher DM, CP, NDF, ADF, and ash contents (Baumgartel *et al.*, 2007). Basalan *et al.* (2011) concluded that sampling time, and possibly, season of harvest may influence the chemical composition of GP. Table 2.1 shows variation in chemical composition of the red and white grape pomaces.

Table 2.1. Nutrient composition (g/kg) of white and red grape pomace

Component	White Pomace	Red Grape Pomace
N	11	17
Dry matter	299.0	348.4
Crude protein	83.1	108.4
Ether extract	48.6	46.2
Neutral detergent fibre	374.9	425.3
Acid detergent fibre	294.4	360.8
Ash	50.3	63.0

Source: Basalan *et al.* (2011)

Red grape pomace is known to have high percentage of tocopherol or vitamin E. The inclusion of GP in feed rations does not only enhance the oxidative stability of the meat and reduce the amount of additives such as vitamin E but also improves meat quality through direct addition of these natural antioxidants, thereby helping to meet consumer demand for healthier meat products (Agustin *et al.*, 2016). Polyphenols in GP enhance the growth of specific beneficial bacteria strains in the intestinal tract while competitively excluding certain pathogenic bacteria (Lipiński *et al.*, 2017).

2.3.1.1 Fibre

Fibre is the main component of dried wine pomace, with concentrations ranging between 43% and 75%. The fibre is mainly constituted of cell wall polysaccharides and lignin. Generally, seeds are richer in fibre than skin, and red wine pomace is richer in fibre when compared to white wine pomace (Gul, 2013). Saura-Calixto *et al.* (1991) reported that acid insoluble lignin is the main component of insoluble fibre in both red and white wine pomace.

In addition, the fibre contains a substantial proportion of tannins and proteins (Arnous & Meyer, 2008).

Broiler chickens have limited ability to utilise diets high in fibre. According to Mpofu *et al.* (2016) birds exposed to high fibre diets tend to have long intestines as an adaptive mechanism to deal with increased amount of fibre. The bird produces digestive enzymes that play a significant role in the digestion of complex feed compounds consumed by chickens into small particles that can be absorbed across the intestinal wall. The utilization of nutrients from the diet is fundamental in the normal functioning of a bird (Bell, 1993).

2.3.2 Potential nutritional implications of fibre

According to Mateos *et al.* (2012) dietary fibre contains diverse polymers with large differences in physicochemical properties that, when included in the diets, result in differences in digestive viscosity, ion-exchange capacity, fermentation capability and bulking effect within the gastrointestinal tract. Poultry require a certain amount of fibre for proper development and physiology of the GIT. Dietary fibre affects GIT development in different ways, depending on the amount and type of fibre used. However, high fibre diets generate physical distension of the walls of the GIT, increasing GIT capacity and gut fill. One of the main advantages of dietary fibre inclusion is its positive effect on gizzard development and functionality. Dietary fibre is necessary to regulate digestion in broilers and laying hens. High starch diets favour fermentation in the small intestines where pathogens can quickly multiply and harm the animal creating a situation when a microbial imbalance occurs. Including dietary fibre aids will support peristalsis, thus moving along the development of the fermentation process into the large intestine and increasing the growth of beneficial bacteria. High polyphenol content and high level of fibre fraction are the major limitations of using GP in broiler diet. Grape pomace contains high level of fibre and polymeric polyphenols as

procyanidins could be bound and precipitated both dietary and endogenous proteins. Polyphenolic compounds could be bound to digestive enzymes and proteins located at the luminal side of the intestinal tract and reduce apparent digestibility of protein in polyphenol-containing diets. Though fibre has been reported to have a beneficial effect on poultry in moderate levels, increasing fibre in the diets might impose a detrimental impact on performance. Grape pomace has potential to serve as an important source of insoluble fibre for functional food development. Inclusion of GP in poultry feed could result in the beneficial effects of dietary fibre and grape polyphenols.

2.3.2.1 Protein

The protein content of wine pomace may range from 6% and 15%, depending on grape variety and harvesting conditions. The amount of protein in the seeds is more than the skin. . Wine pomace has an amino acid profile similar to that of cereals, being rich in glutamic acid and aspartic acid and deficient in tryptophan and sulphur-containing amino acids. Furthermore, the skin protein content is rich in alanine and lysine, a fact that is not realized in the proteins of seeds. Grape seeds are not considered as an important protein source as legumes and nuts, although grape seeds contain 11–13% proteins (Goni *et al.*, 2005). The total protein content and the amino acid composition of grape seed protein may vary significantly depending on the variety of grape, location and fertilisation conditions. However, grape seed protein was considered as non-digestible or resistant protein (Saura-Calixto *et al.*, 1991). The complexation between protein and tannin limited the digestibility of the grape seed protein because tannin is believed to be a potent inhibitor of digestive enzymes (Alipour & Rouzbehan, 2010).

2.3.2.2 Lipids

The major lipid contribution in wine pomace is from the seeds. Seeds from wine pomace have lipid contents ranging between 14 and 17% (Gul *et al.*, 2013). Furthermore, the lipid fraction presents an interesting fatty acid profile that is rich in polyunsaturated fatty acids and monounsaturated fatty acids, with low levels of saturated fatty acids. Linoleic acid, oleic acid and palmitic acid are the predominant fatty acids in grape seed oil (Fernandes, 2013).

2.3.2.3 Minerals

The mineral content of wine pomace may present even wider variations than in the case of the other components due to the strong influence of climatic conditions, viticultural practices, and the winemaking process. The type and mainly the duration of maceration processes have a strong influence on the extraction and reabsorption of minerals during the winemaking, notably affecting the mineral content remaining in wine pomace (Gayon, 2006). Minerals in grapes are usually classified in groups depending on their mobility in phloem. Potassium, phosphorus, sulfur, and magnesium show high mobility and are accumulated and mainly localized in the skin of the grape berry during ripening. Consequently, grape skins have higher levels compared to grape seeds, mainly due to their high content of potassium salts localized in grape skins, specifically in the hypodermal cells (Rogiers, 2006). In contrast, seeds are the largest reservoir of calcium, phosphorus, sulfur, and magnesium (Gul, 2013). The most abundant potassium salts are tartrate, mainly potassium bitartrate ($KC_4H_5O_6$). Tartrate salts are mainly in the form of potassium bitartrate ($KC_4H_5O_6$), although calcium tartrate ($CaC_4H_6O_6$) can also be in significant concentrations (Rice, 1976; Nurgel & Canbas, 1998).

2.3.2.4 Phenolic compounds

The phenolic composition of grape pomace has been widely described (Kammerer *et al.*, 2004; Molina & Castro, 2013) with notable qualitative and quantitative differences. According to Gharras (2009) phenols are classified according to their chemical structure and molecular weight in the following groups: simple phenols (mainly C_6 - C_1 and C_6-C_3), flavonoids (C_6-C_3-C_6 and oligomers), polymeric compounds (including hydrolyzable and condensed tannins, lignin) and miscellaneous phenol groups with different structures (xanthones, stilbenes, beta-cyanines,).

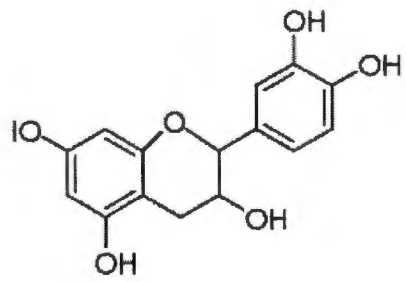

Figure 2.1. Chemical structure of phenolic compounds in grape pomace (Source: James *et al.*, 2006)

Skins from wine pomace are richer in phenolic acids than from white grapes. Grape skins are rich in hydroxycinnamic acids (C_6-C_3) and especially rich in tartaric esters of these acids, mainly caftaric acid and coutaric acid followed by fertaric acid. In the contrary, seeds are rich in gallic acid and protocatechuic acid (Kammerer *et al.*, 2004). The presence of tartaric ester in the skins is associated with the pulp remains sticking to them, due to pulps' highest levels of those types of compounds (Kammerer *et al.*, 2004). Flavonoids are a very extensive group of phenolic compounds that include a wide range of different families or subgroups, mainly

differentiated by the degree of oxidation of their oxygenated heterocycle. Anthocyanins (in red pomace) and flavanols are the most abundant in wine pomace. According to the normal composition of *Vitis vinifera* red varieties, the predominant anthocyanin is *malvidin-3-O-glucoside* that is usually followed by peonidin, petunidin, or delphinidin-3- glucoside depending on the grape variety (Kammerer *et al.*, 2004)

The absence of anthocyanins in white grapes leaves flavanols as the most abundant phenols in white wine pomace. Flavanols are mainly located in the seeds, whose levels range from 56% to 65% of the total flavanols of grapes against 14% to 21% present in grape skins. The seeds are rich in gallocatechins (Montealegre *et al.*, 2006), whereas the presence of epigallocatechin (tri-hydroxyl catechin) has only been described in skins (Bailon *et al.*, 1994). In addition, oligomers (from 2 to 5 units) and polymers of flavanols are in relevant concentrations, with significant predominance of type-B proanthocyanidins. Proanthocyanidins from seed wine pomace have a lower average degree of polymerization (10 to 20 units) than the skins (25 to 35 units) (Ky *et al.*, 2014). Oligomers and polymers with low levels of solubility are not extracted during winemaking processes and remain in the wine pomace. The clear relevance of quercetin 3-O-glucuronide in comparison with other flavonols has been described in the wine pomace of some specific varieties (Ruberto *et al.*, 2007); although other studies indicated similar concentrations of quercetin 3-O-glucuronide and quercetin 3-O-glucoside with slight differences between grape varieties (Kammerer *et al.*, 2004).

2.3.3 Anti-nutritional factors in grape pomace

According to Gemede and Ratta (2014), anti-nutritional factors are compounds that when present in animal feed reduce the availability of one or more nutrients, which result in the reduction of feed utilization and/or feed intake. Grape pomace contains anti-nutritional

factors such as tannins and fibre that may cause a decrease to its feeding value. Tannins are defined as phenolic compounds of high molecular weight.

According to Ping *et al.* (2011) tannins are astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins are tentatively classified into two classes: hydrolysable and condensed tannins, although there are tannins known to have components of both hydrolysable and condensed tannins. These compounds are considered to have both adverse and beneficial effects depending on their concentration, molecular weight, animal species, physiological state of the animal and composition of the diet (Ping *et al.*, 2011). Hydrolysable tannins are identified as polyesters of phenolic acids such as gallic acid, hexahydroxydiphenic acid and/or their derivatives and D-glucose or quinic acid. The condensed tannins are categorised as polymers of flavan-3-ols, flavan-3, 4-diols or related flavanol residues linked via carbon–carbon bonds. Condensed tannins do not have a carbohydrate core found in hydrolysable tannins. According to Bosso *et al.* (2016) tannins have been closely linked with plant defence mechanisms against ruminant animals, birds, and insects. They act as anti-nutritional factors when included in the diet of animals. Tannins have the ability to bind dietary protein as well digestive enzymes making the proteins unavailable to the animal. If tannin concentration in the diet becomes too high, enzyme activities and intestinal digestion may be depressed to the nutritional detriment of the target animal (de Sales *et al.*, 2018).

2.4 Dietary grape pomace: Nutrient utilization and carcass and meat quality traits in birds

Recent studies have documented that the amount of non-absorbable polyphenols reaching the colon is extremely high and microbe-derived phenolic metabolites excreted in urine represent the largest proportion of polyphenol intake (Monagas *et al.*, 2010). Birds lack a specific urinary excretion system and consequently, the excreta of chicks are composed of both urine and undigested dietary components. Chamorro *et al.* (2012) concluded that birds fed GP diets showed a higher ileal and fecal polyphenol content and the inclusion of tannase in GP diets increased ileal polyphenol content and did not affect the fecal polyphenol content. De Sales *et al.* (2018) stated that dietary polyphenol-rich grape products are effective in increasing the growth of specific beneficial intestinal bacteria while competitively excluding certain pathogenic bacteria. Previous studies have reported a negative impact of fibre sources on daily feed intake, growth performance, and nutrient digestibility (Sklan *et al.*, 2003). Ebrahimzadeh *et al.* (2018) reported that addition of up to 10% GP in broiler chicken diets had no effect on average daily feed intake. Kara *et al.* (2012) concluded that grape pomace addition of up to 6 % in laying hen diets did not affect their feed intake. In the contrast, Sayago-Ayerdi *et al.* (2009) stated that inclusion of GP over 6 % reduce feed intake. Therefore, nutrient digestion and the growth performance of chicken could be impaired by incorporation of GP at high doses in chicken diets.

Carcass classification and grading systems have been developed to describe the yield and features of carcasses, which are useful for trading and pricing purposes (Soji & Muchenje, 2016). Chicken meat is rich in polyunsaturated fatty acids, especially n-3 PUFA that are beneficial for the human health (Popova, 2016). Poultry meat quality is affected by the genotype, diet, age at slaughter and motor activity of birds, and their adaptation for outdoor

production (Castellini *et al.*, 2002). A study by Chamorro *et al.* (2012) concluded that the oxidative stability of thigh meat after 1 and 4 days of refrigerated storage increased with the dietary addition of α-tocopheryl acetate (αT) and GP, and was worsened when GP was supplemented with carbohydrases. Birds fed αT and GP diets showed higher meat polyunsaturated fatty acid content, while monounsaturated fatty acid content was reduced. Brenes *et al.* (2008) and Goni *et al.* (2007) indicated that the intake of grape pomace increases the antioxidant capacity in breast and thigh meat of broiler chickens. Enhanced antioxidant capability in the muscle tends to improve meat quality and extend the shelf life (Tavárez *et al.*, 2011). Brenes *et al.* (2016) concluded that the inclusion of GP in feed rations enhances the oxidative stability of the meat and reduces the amount of additives required, such as vitamin E. The application of antioxidants directly into meat and meat products with grape by-products improve their oxidative stability, the overall sensory and nutritional quality of meat and meat products, and hence their shelf life (Brenes *et al.*, 2016).

2.5 Amelioration of dietary tannins

A number of methods have been used to reduce or eliminate the detrimental and toxic effects of tannins in order to improve the nutritive value of tannin-rich feedstuffs. These methods include the use of tannin-binding agents (polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, acetic acid and sodium hydroxide), enzymes, heating, soaking in water and drying, wood ash, chopping and storage, urea and solid state fermentation (Ben Salem *et al.*, 2000; Silanikove *et al.*, 2001; Makkar, 2003). The methods are commonly grouped into physical and chemical methods.

2.5.1 Physical methods

According to Ben Salem *et al.* (2000) drying reduces levels of condensed tannins in polyphenolic plants. Indeed, drying cassava and Leucaena leaves at 90 °C for 24 hours decreased tannin content (Ben Salem *et al.*, 2000). Tannin content can be reduced by storing fresh (40 % moisture leaves), chopped leaves at 37 °C prior feeding (Ben Salem *et al.*, 2000). Grinding and chopping decrease the negative effects of tannins in leaf meals (Wina *et al.*, 2005). Medugu *et al.* (2012) recommended soaking amongst other methods as reducing the effects of tannins. According to Hlatini and Chimonyo (2015) other physical methods do not seem to be economically viable and are less effective due to intensive labour and time requirements. However, chopping of fresh leaves, water soaking and then storage can be of practical use to the farmer, as it requires only minor changes in normal farm practices (Bhat *et al.*, 2013).

2.5.2 Chemical methods

Several chemical methods from urea to tannin-binding agents for inactivating tannins have been used (Kyarisiima *et al.*, 2004; Alipour & Rouzbehan, 2007). Kyarisiimamla *et al.* (2004) reported that treating sorghum with wood ash reduces the level of tannins. The wood ash is an inexpensive and locally available source of alkali that is recommended to inactivate tannins. Mlambo *et al.* (2011) concluded that wood ash has the potential to improve the nutritive value of underutilised, high-tannin feeds under smallholder conditions in Zimbabwe. The use of urea to neutralize tannin-rich leaf meals is recommended and also gives extra nitrogen to the animals (Sahnoune *et al.*, 1991). Moreover, addition of urea is effective due to the higher pH caused by evolution of ammonia. Tannin-binding agents and enzymes are also used to neutralize anti-nutritive factors (Schons *et al.*, 2012). Chamoro *et al.* (2015) evaluated the supplementation of carbohydrase complex and tannase enzyme on chicks fed grape

pomace and concluded that the inclusion of tannase in diets containing GP increased the amount of total polyphenol released in the intestine, but did not improve the stability of meat to lipid oxidation. Hlatini & Chimonyo (2015) evaluated the influence of polyethylene glycol inclusion in *Vachellia tortilis* leaf meal on nitrogen balance in growing pigs and concluded that the inclusion of increasing levels of PEG in *V. tortilis* leaf meal-based diet showed a linear response to digestion and the use of PEG to neutralize the negative effects of polyphenolic compounds was recommended. Polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, charcoal and sodium hydroxide are common examples of tannin-binding agents. Challenges of chemical methods include the loss of soluble nutrients; analyses and laboratory work are costly to resource-poor farmers. There is little information on the use of tannin-binding agent such polyethylene glycol to enhance the feed value of polyphenolic plants such as GP for broiler chickens.

2.5.3 Polyethylene glycol as a tannin-inactivating agent

According to Besharati and Taghizadeh (2011) polyethylene glycol (PEG), also referred to as polyethylene oxide (PEO), is a condensation polymer of ethylene oxide and water that has several chemical properties that make it useful for biological, chemical and pharmaceutical applications. Polyethylene glycol can be easily synthesized by the anionic ring opening polymerization of ethylene oxide into a range molecular weights and a variety of end groups, which enables PEG to be used in multiple research applications. Polyethylene glycols of different molecular weight are available commercially, and their molecular weight ranges from as little as 200 (g/mol). Makkar *et al.* (1995) reported that PEG 4000 and 6000 has more capacity to bind with tannins than PEG of various other molecular weights at near neutral pH.

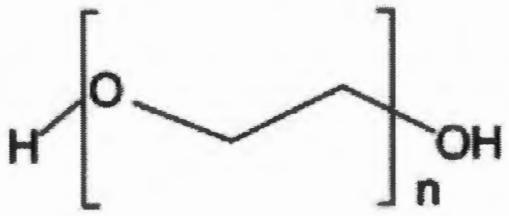

Figure 2.2. Chemical structure of PEG 4000 (Source: Chen *et al.*, 2012)

The use of PEG increases the availability of plant proteins for digestion and absorption by binding to tannins and releasing them from protein-tannin complexes (Besharati & Taghizadeh, 2011). Polyethylene glycol is a suitable material for biological applications because it does not initiate an immune response in animals. Polyethylene glycol has numerous properties such the ability to dissolve in water, viscosity, hygroscopicity and solubility on inorganic solvents (Turner *et al.*, 2011). Ben Salem *et al.* (2000) reported that PEG is used to increase availability of proteins from ingested tannin-containing feed in livestock.

2.5.3.1 Effects of polyethylene glycol

Besharati and Taghizadeh (2011) reported that PEG breaks already formed tannin-protein complexes due to its high affinity for tannins. It deactivates tannins over a wide pH range of 2 to 8.5. Adequate oxygen molecules from water-soluble PEG form hydrogen bonds with phenolic and hydroxyl group in tannins (Hlatini & Chimonyo, 2015). This phenomenon makes protein and other nutrients available for utilization, and increases the voluntary intake of tannin-rich feedstuffs (Mantz, 2008). Previous research has found that PEG binds with the condensed tannins (found in the leaves of some plants including mulga) allowing more protein to be digested by ruminants (Mantz *et al.*, 2008). Addition of PEG in diets containing

high levels of tannins improves the feed value of such diets. Henkin *et al.* (2009) demonstrated that PEG supplementation affected cow grazing behaviour (increased foraging time and daily foraging distance), increased usage of woody species containing CT but not average cow live weight. Polyethylene glycol alone may not be enough to improve animal performance. Previous studies with sheep fed mulga found that supplements with PEG, true protein, nitrogen, phosphorus and sulphur markedly improved dry matter intake and digestibility and wool growth (Pritchard *et al.*, 1992) and live weight gain (Miller, 2003). However, similar studies in broilers fed with red grape pomace have not been carried out despite the possible advantages of improving intake and utilization of red grape pomace.

2.5.3.2 Constraints to utilization of polyethylene glycol

The general characteristics to consider when deciding to use PEG include functionality, reactivity, polymer architecture and molecular weight. The PEG must have a large number of oxygen atoms and it should contain sufficient oxygen molecules in a chain to form strong bonds with phenolics and hydroxyl groups of tannins (Hlatini & Chimonyo, 2015). Turner *et al.* (2011) discovered that the potentially unfavourable effects that might be caused by PEG can be divided into several groups; adverse side effects in the body can be motivated by the polymer itself or by side products formed during synthesis that lead to hypersensitivity. When excess amount is given, unexpected changes in the body of animal can occur with PEG-based carriers. The PEG requires some skills and knowledge to use without compromising livestock. The PEG might be administered to animals in different ways such as spraying leaves, oral dosage and mixed with diet (Ben Salem *et al.*, 2000). Application of PEG by spraying the standing plants prior utilization requires a lot of labour and its time consuming, while mixing with diets is preferred. Oral dosage may result in a slower onset of action, irritation and stress to animals (Turner *et al.*, 2011). However, spraying and oral dosage both

require moderate technical skill. There is very little information in literature concerning potential enhancement of feed value of GP for broiler chickens using PEG. Polyethylene glycol increases digestibility, utilization and absorption of nutrients in polyphenolic rich plants. As such, there is a need to explore the potential nutritional benefits of incorporating this tannin-binding agent to chicken diets. The PEG does not affect digestion processes, intake and growth performance of animals fed on diets without polyphenolic compounds (Getachew *et al.*, 2000).

2.6 Enzymes as additives in poultry diets

According to Khattak *et al.* (2006) enzymes are biological catalyst composed of amino acids with vitamins and minerals. They bring about biochemical reactions without themselves undergoing any change (Farrell *et al.*, 1993). Enzymes are produced in every living organism from the highest developed animals and plants to the simplest unicellular forms of life, as they are essential for metabolic process. The benefits of using enzymes in poultry diets include not only enhanced bird performance and feed conversion but also less environmental problems due to reduced output of excreta. Poultry naturally produces enzymes to aid the digestion of feed nutrients (Khattak *et al.*, 2014). However, they do not produce enzymes required to break down fibre completely and need exogenous enzymes in feed to aid digestion. Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, often because the animal cannot produce the necessary enzyme to degrade them (Campbell *et al.*, 1989). Du *et al.* (2011) optimized the extraction of soluble dietary fibre from grape pomace. These enzymes come from microorganisms that are carefully selected for the task and grown under controlled conditions (Wallis, 1996). Enzymes are one of the many types of proteins in biological systems. Another important feature of enzymes is that the rate of an enzyme catalyzed reaction increases with increasing

substrate concentration, to the point where there is no further response and the enzyme is said to be saturated. Therefore, there is a need to match the amount of enzyme with the quantity of substrate (Acamovic & McCleary, 1996). Their essential characteristic is to catalyze the rate of a reaction but inot themselves being altered by it. According to Choct *et al.* (1995) benefits of using feed enzymes to poultry diets include; reduction in digesta viscosity, enhanced digestion and absorption of nutrients especially fat and protein, improved Apparent Metabolizable Energy (AME) value of the diet, increased feed intake, weight gain, and feed–gain ratio, reduced beak impaction and vent plugging, decreased size of gastrointestinal tract, altered population of microorganisms in gastrointestinal tract, reduced water intake, reduced water content of excreta, reduced production of ammonia from excreta, reduced output of excreta, including reduced N and P (Campbell *et al.*, 1989). Enzymes are involved in all anabolic and catabolic pathways of digestion and metabolism. Enzymes tend to be very specific catalysts that act on one or, at most, a limited group of compounds known as substrates. The use of enzymes in animal feed is of great importance. Consistent increase in the price of feed ingredients has been a major constraint in most of the developing countries. As a consequence cheaper and non-conventional feed ingredients have to be used which contain higher percentage of non-starch polysaccharides (soluble and insoluble/crude fibre) along with starch.

2.6.1 Carbohydrase enzyme complex

Carbohydrase enzymes are considered for use in poultry feeds and have been proven effective in increasing the amount of energy available from feed ingredients. Key carbohydrase enzymes include amylase and xylanase and are used to improve the digestibility of carbohydrates in feed ingredients. This improved digestibility increases the availability of energy in the small intestine to help promote growth and other productive processes. A recent

in vitro study (Chamorro *et al.*, 2012) reported that the addition of carbohydrases (pectinases and cellulases) and tannase released polyphenols and polysaccharides entrapped in grape pomace cell wall thus increasing its antioxidant activity. According to Chamorro *et al.* (2017), the addition of enzymes (mainly tannase) hydrolyzes the polymeric tannin structures into smaller catechins, and also promotes a lower digestibility of the monomeric and dimeric catechins suggesting that polymeric structures might favour the intestinal utilization of these catechins. Cell wall-degrading enzymes can improve the extraction of phenols during digestion. The addition of carbohydrases to grape pomace, either alone or in combination, degrades the cell wall polysaccharides, increasing the content of monosaccharides (Chamorro *et al.*, 2012). The effectiveness and activities of these enzymes can be influenced by several factors related to the enzyme source, biochemical characteristics, diet, animal and environmental conditions. Carbohydrases are known to modify gut microbiota of chickens by increasing the rate of digestion and limiting the amounts of substrates available to the microflora (Adeola & Cowieson, 2011). Grape pomace has high dietary fibre hence treating grape pomace with fibrolytic enzyme would break down the cell wall structures of GP and reduce anti-nutritive effects while improving broiler performance.

2.6.1.1 Viscozyme®

Viscozyme® is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase (Gama *et al.*, 2015). The enzyme preparation is produced from a selected strain of *Aspergillus aculeatus*. Viscozyme® was shown to be an effective enzyme for the extraction of polyphenols. It also acts on branched pectin-like substances found in plant cell walls. Viscozyme® is a clear brown liquid with a density of approximately 1.2 g/ml. It is a special enzyme preparation used in the breakdown of cell walls for the extraction of useful components from plant tissue (Perussello

et al., 2017). The optimal conditions for Viscozyme® with its several and complex activities are a pH range of 3.3-5.5 and a temperature of 25-55°C. Viscozyme® complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO committee. Viscozyme® is non-flammable, completely miscible with water and safe when used according to directions.

2.6.1.1.1 Cellulase

Cellulase is a class of enzymes produced by the fungi bacteria and protozoans that generate cellulolysis (Watanabe & Tokuda, 2001). Cellulases break down the cellulose molecule into monosaccharides (simple sugars) such as beta-glucose, or shorter polysaccharides and oligosaccharides.

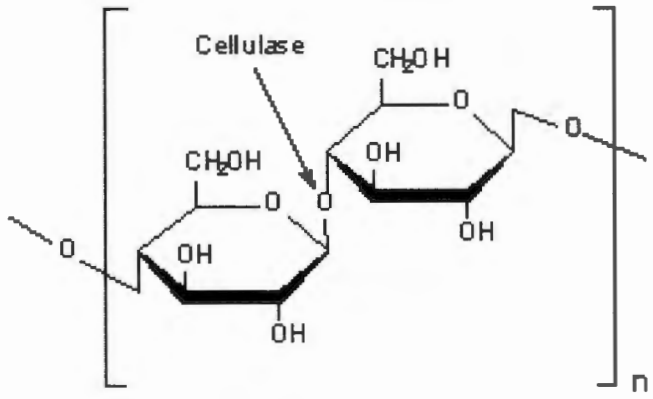

Figure 2.3. Chemical structure of cellulase in grape pomce (Source: James *et al.*, 2006)

Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions. Since cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides such as starch.

2.6.1.1.2 Xylanase

Xylanases are glycosidases (O-glycoside hydrolases, EC no: 3.2.1.x) which catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan (Collins *et al.*, 2005). They are a widespread group of enzymes, involved in the production of xylose, a primary carbon source for cell metabolism and in plant cell infection by plant pathogens, and are produced by a plethora of organisms including bacteria, algae, fungi, protozoa, gastropods and anthropods. Xylan is the major hemicellulosic and is a heteropolysaccharide containing O-acetyl, arabinosyl and 4-O-methyl-d-glucuronic acid substituents. It is the next most abundant renewable polysaccharide after cellulose. Xylanases and associated debranching enzymes produced by a variety of microorganisms including bacteria, actinomycetes, yeast and fungi bring hydrolysis of hemicelluloses (Walia *et al.*, 2017).

2.6.1.1.3 Glucanase

Glucans are glucose polymers categorised according to inter chain linkage (Moreno-Mendieta *et al.*, 2017). Glucanase hydrolyzes the β-1,3-gylocsidic bonds found in beta-glucans cell walls. As they perform hydrolysis of the glucosidic bond, they hydrolase. Glucans play a pivotal role in the biomedical and pharmaceutical industries due to their anticoagulant, antioxidant and anti-inflammatory properties (Kagimura *et al.*, 2015).

2.6.1.1.4 Hemicellulase

Hemicelluloses are polysaccharides in plant cell walls that have beta-(1-->4)-linked backbones with an equatorial configuration (Peng *et al.*, 2015). Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and beta-(1-->3,1-->4)-glucan. Hemicelluloses act as linkers between lignin and cellulose.

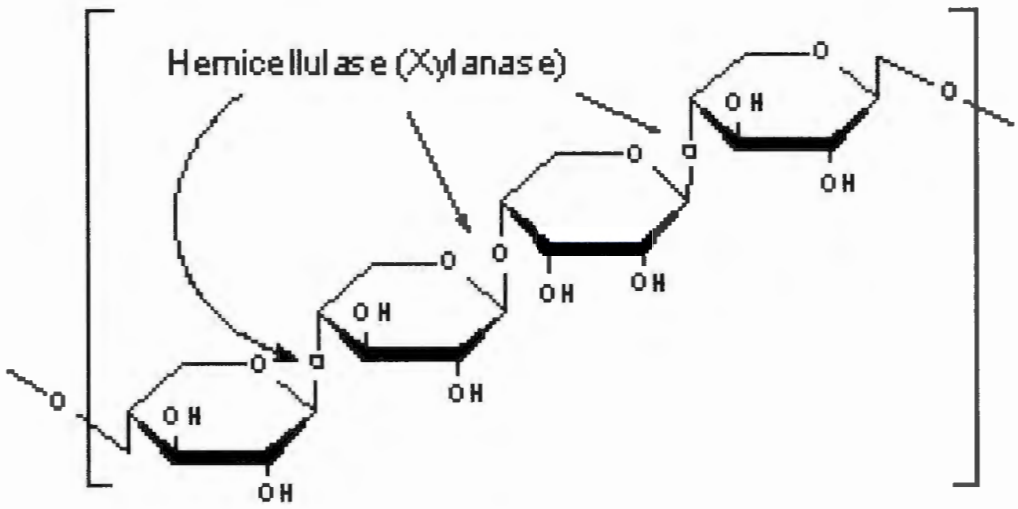

Figure 2.4. Chemical structure of hemicellulase present in grape pomace (Source: James *et al.*, 2006)

2.6.2 Phytases and Proteases

Phytase is a phosphate enzyme that acts on monoester and is capable of hydrolyzing phytic acid to produce inorganic orthophosphate and liberate myo-inositol (Irving & Cosgrove, 1972). Phytase works by releasing some of the non-digestible phosphorus (and other nutrients) found in commonly used feed ingredients and making the nutrients available for productive purposes. Phytase is a proven technology used to reduce feed cost by reducing inorganic phosphorus supplementation and has the added benefit of decreasing phosphorus excretion in manure. Phytases are derived from plant material, animals and microorganisms, however, the microbial derived phytase are more reliable. Phytase effectiveness is influences by pre-treatment and inclusion levels. The phytase enzyme frees the phosphorus in feedstuffs and also drives the release of other minerals (e.g. Ca, Mg), as well as proteins and amino acids bound to phytate. Thus, by releasing bound phosphorus in feed ingredients, phytase reduces the quantity of inorganic phosphorus needed in diets, makes more phosphorus available for the bird, and decreases the amount excreted into the environment.

Protease enzymes are a group of enzymes that break down molecules of proteins into shorter peptides. They are known to act on the cell wall by removing the structural proteins to allow faster digestibility. They are classified into six groups based on their catalytic mechanism: aspartic, glutamic, metalloprotease, cysteine, serine and threonine (Rawlings *et al.*, 2004). Protease enzymes have the potential to improve protein digestibility. The addition of a protease to a feed can result in improved amino acid digestibility across various protein sources and minimize the impact of anti-nutritional factors. A protease can degrade anti-nutritional factors and allergenic proteins in feedstuffs. By improving protein digestibility, proteases reduce undigested protein entering hind gastro intestinal tract, reduce protein fermentation in the large intestine and hence improve gut health. Protease is a protein digesting enzyme that breaks down storage proteins binding starch within feed ingredients. This makes the energy from protein bound starch available to the bird to be used for productive purposes. Proteases are also effective in releasing protein anti-nutrients found in ingredients proteases makes proteins more available. The improvement of feed efficiency is an important issue in animal nutrition because of the need to reduce environmental pollution from farm animals and to decrease production costs. Feedstuffs contain certain compounds that animals cannot digest or that interfere with the animals' digestive system. A frequent reason for these problems is that the animals are unable to produce the necessary enzymes to degrade the compounds (Khattak *et al.*, 2006). According to Blazek (2008), the ability of protease enzymes to coagulate protein diets depends on the type of dietary protein and the nature of the enzyme.

2.7 Benefits of using enzymes in poultry feed

According to Ravindran (2013), use of enzymes increases the range of feedstuffs that can be used and increases flexibility in feed formulations by reducing or removing the constraint on the inclusion limit of poorly digested ingredients. Enzymes have been approved for use in poultry feed because they are natural products of fermentation and, therefore, pose no threat to the animal or the consumer (Campbell *et al.*, 1989). Use of enzymes also reduces variability in the nutritive value between batches of ingredients. Enzyme supplementation uplifts the value of poor samples and reduces the variation between good and poor quality samples of a given ingredient (Saleh *et al.*, 2003). This effect, in turn, improves the degree of precision of feed formulation. Because of improved digestion and fewer amounts of undigested nutrients reaching the lower gut, as well as a shift in gut flora toward favorable bacterial species, gut health is improved. A related outcome is the protective effect on the overall health of the bird, due in part to the influence of flora on immune function. Enzymes will not only enable poultry producers to use new feedstuffs economically but will also reduce environmental pollution associated with animal production (Choct *et al.* 1995). Consequently, in addition to improved poultry production, feed enzymes can have a positive impact on the environment. Enzymes also cause improved intestinal morphology and integrity resulting in enhanced digestion and absorption of dietary components (Classen *et al.*, 1995). Benefits of using feed enzymes to poultry diets include; reduction in digesta viscosity, enhanced digestion and absorption of nutrients especially fat and protein, improved Apparent Metabolizable Energy (AME) value of the diet, increased feed intake, weight gain, and feed–gain ratio, reduced beak impaction and vent plugging, decreased size of gastrointestinal tract, altered population of microorganisms in gastrointestinal tract, reduced water intake, reduced water content of excreta, reduced production of ammonia from excreta, reduced output of excreta, including reduced N and P (Campbell *et al.*, 1989; Jansson *et al.*,

1990). In summary, the benefits of exogenous enzymes go far beyond just improving nutrient digestion and have implications in the on-going changes in global poultry production in terms of environment, gut health, bird welfare, and sustainability. It must be recognized that different feed enzymes will have different modes of action. Despite their increasing acceptance as feed additives, the exact mode(s) of action of feed enzymes remains to be elucidated. The ultimate aim of adding enzymes is to improve bird performance and profitability through enhanced digestion of dietary components (protein, amino acids, starch, lipids, and energy) in ingredients. Addition of enzymes to the poultry diets has a positive response on the digestibility of feeds and leads to better performance. In addition, supplementation of enzymes can improve the productive value of commercial feeds and allow greater flexibility in feed formulation.

2.7.1 Factors affecting enzyme effectiveness

The degree of improvement obtained by adding enzymes to the diet depends on many factors (Bedford, 2012), including the type and amount of substrate in the diet; the level of antinutritive factor, which can vary within a given feedstuff (e.g. low- versus high-ß-glucan barley); the spectrum and concentration of enzymes used; the type of animal (poultry tend to be more responsive to enzyme treatment than pigs); and the age of the animal (young animals tend to respond better to enzymes than older animals); type of gut micro flora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the micro flora in their intestines, have a greater capacity to deal with negative viscosity effects (Allen *et al.*, 1995). Typically, enzymes added to layer feeds had little effect on egg mass but improved feed efficiency (Benabdeljelil & Arbaoui, 1994) and energy utilization. Wyatt and Goodman (1993) reported that corn-fed layers exhibited higher feed efficiency than those fed enzyme supplemented barley-based diets. Nevertheless, enzyme supplementation improved

the utilization of barley diets. Adding enzymes to both wheat and barley-based diets has been shown to reduce the moisture content of fecal matter in layers (Marquardt *et al.*, 1994). The use of enzymes as a feed additive has rapidly expanded. In the last decade, extensive studies have been conducted to investigate the effects of feeding exogenous enzymes on the performance of poultry. A study by Chamorro *et al.* (2014) on the influence of dietary enzyme addition on polyphenol utiliation and meat lipid oxidation of chicks fed grape pomace concluded that the inclusion of tannase in diets containing GP increased the amount of total polyphenol released in the intestine, but did not improve the stability of meat to lipid oxidation. Recent results from Ebrahimzadeh *et al.* (2017) showed that dietary inclusion of 10%GP with or without tannase enzyme treatment did not affect chick growth performance. These findings show that the inclusion of enzymes in diets containing GP increased the amount of total polyphenol released in the intestine, although this effect was not accompanied by an increase in performance.

2.8 Poultry responses to high levels of dietary fibre

Dietary fibre is defined as the part of plant material consisting mainly of cellulosic and non-cellulosic polysaccharides, and a non-carbohydrate component, lignin. These components are extremely resistant to hydrolysis by alimentary enzymes and cannot, therefore, be digested or absorbed in the blood stream. Fibre has also been regarded as nutrient diluent in poultry (Angkanaporn *et al.*, 1994) and higher fibre in feed ingredients has been shown to have negative effects on digestion and absorption of nutrients in chickens (Krogdahl, 1986). However, previous experiments have indicated that performance does not decrease when feed ingredients high in fibre are included at sensible levels in both layer and broiler diets despite the reduction in nutrient concentration of the diet (Hetland & Svihus, 2001). In the past, poultry diets have been formulated with low fibre concentrations because the addition of

higher fibre ingredients decreases feed efficiency (Longe & Ogedegbe, 1989). There have been several experiments involving the use of higher fibre ingredients in adult laying hens' diets and 50-200 g/kg of DDGS have been suggested to have no effects on production (Lumpkins *et al.*, 2005). However, there are few reports concerning the use of high fibre ingredients in layer chicks. Masa'deh *et al.* (2012) fed layer chicks up to 125.0 g/kg of corn DDGS without negative performance results. Higher fibre concentrations in chicken diets can have negative effects on nutrient digestion and absorption (Walugembe, 2013) and may subsequently affect performance. Traditionally, dietary fibre has been considered a diluent of the diet and, often, an antinutritional factor (Mateos *et al.*, 2012). However, moderate amounts of fibre might improve the development of organs, enzyme production, and nutrient digestibility in poultry. Some of these effects are a consequence of better gizzard function, with an increase in gastro-duodenal refluxes that facilitate the contact between nutrients and digestive enzymes. These effects often result in improved growth and animal health, but the potential benefits depend to a great extent on the physicochemical characteristics of the fibre source.

2.9 Haematological parameters of broiler chickens

The use of blood examination as a way of assessing the health status of animals has been documented (Muhammad & Oloyede, 2009). Biochemical changes as a result of toxins have effects on haematological parameters. Haematological parameters have commonly been used as indicators of physiological conditions and nutritional deficiency in chickens. Apart from the physiological and nutritional aspects, haematological variables can also be used as an indicator of health in birds. The purpose of investigating blood composition is to be able to distinguish the normal state from the state of stress. The stress factors could be nutritional, environment or physical. Indeed, several factors have been shown to influence the

haematological variables including species, age, sex, environment, nutrition, infection and physiological conditions. With regard to nutrition, this factor seems to be a determinant factor in the production of blood constituents (such as haemoglobin, erythrocyte and leukocyte) (Karadeniz *et al.*, 2008) Haematological parameters provide the opportunity to evaluate the presence of several metabolites and other constituents in the body of broiler chickens. Changes in the constituent compounds of blood when compared to normal values could serve as a reflector of the metabolic stage of an animal as well as quality of feed. If farm animals are offered a diet containing high amounts of toxic substances, their health is compromised causing acute histopathological and damage of body organs such as the liver, kidney and spleen (Ewuola *et al.*, 2003). The haematological parameters are beneficial in the diagnosis of many diseases, identification of disorder and examination of damaged organs (Onyeyili *et al.*, 1992). The influence of grape pomace diets on haematological parameters of broiler chickens is limited. This study will clarify how the inclusion of grape pomace in chicken diets would influence the haematological parameters of broiler chickens given that the pomace contains antinutritional compounds such as tannins and fibre.

2.10 Serum biochemical parameters of broiler chickens

Serum biochemistry refers to the chemical components of serum, which include proteins, enzymes, lipids, minerals, glucose and hormones. Serum proteins are synthesized in the liver and they maintain blood volume through the colloidal osmotic effect, buffer blood pH, transport hormones and drugs, participate in cell coagulation, catalyze chemical reactions (enzymes), regulate the metabolism (hormones), and participate in the body defence against foreign matter (Melillo, 2013). Determining serum biochemical parameters provides information about the status of organs and tissues in the body as well as the metabolic state of the animal. Washington and Van Hoosier (2012) stated that when a test result is

abnormal, it might indicate that the animal is sick or undernourished. Further assessment of the test results may offer clues about which organ system is affected and also the nature and severity of the disorder.

The two main types of protein found in blood are called albumin and globulin. These proteins can be measured individually or combined into a single test called total protein. According to Evans (2009), albumin levels can indicate if an animal is dehydrated and provides information about the functionality of the liver, kidneys, and digestive system. Globulin levels reflect underlying inflammation and antibody production. According to McLaughlin and Fish (1994) increased levels of globulins are associated with infectious diseases, immune-mediated disease and some types of cancer. Calcium and phosphorus are present in tiny amounts in blood and their inconsistency may be associated with a variety of diseases or conditions. For example, persistently high calcium levels may indicate kidney disease, cancer, or disease of the parathyroid glands, meanwhile low calcium levels may be due to pancreatitis, antifreeze poisoning, or disease of the parathyroid gland. High phosphorus levels are associated with kidney failure and some nutritional problems (Evans, 2009). Low phosphorous level can occur with dietary problems, gastrointestinal disease, and kidney disease. Cholesterol is produced in the liver as part of fat metabolism. Increases in cholesterol are associated with hormonal and metabolic diseases, liver disease and kidney disease. Serum biochemical parameters serve as a practical diagnostic tool for evaluating and monitoring the pathological and physiological conditions as well as the health status of farm animals. However, literature reveals very little about the serum biochemical profiling of broiler chickens fed grape pomace diets.

2.10.1 Liver enzymes that indicate toxicity

Metabolic disorders are arisen by a failure either of a hormone or an enzyme system caused by the nutritional, environmental, management and genetic factors. The liver contains enzymes such as Alanine transaminase (ALT) and Alkaline phosphate (ALP), these enzymes are released into the bloodstream when damaged (Sherwin, 2003). The ALP is an enzyme responsible for dephosphorylation of a substrate therefore it is produced in all types of tissues in the body, but it gets activated in alkaline pH. Therefore, elevated levels of ALP can be mostly seen in liver damages. The ALT enzyme is found in highest amount in liver and is used to identify acute liver failures (Orlewick & Vovchuk, 2012) as the enzyme is released into the serum immediately after a hepatocellular damage. Elevation of these serum enzymes occur with conditions of altered hepatocellular membrane permeability either due to circulatory hypoxia, exposure to toxins and toxaemia, inflammation, metabolic disorders or proliferation of the hepatocyte.

2.11 Nutrition and poultry meat quality

Poultry meat quality has received considerable attention recently due to the emergence of problems associated with poor water holding capacity, poor texture and pale color. The meat has also rightly gained significant market popularity due to it being a lean meat with a favourable (unsaturated) fatty acid profile. Nutritional management has become an integral part of poultry production (Santiago, 2002). The increasing demand for white meat and the continuous improvements in the genetic potential of commercial lines has resulted in important changes in nutritional management of birds. During recent years, it has become a common practice to grow birds under high protein diets in an attempt to maximize growth and production of breast meat. However, while the economic benefits of these changes are obvious in terms of meat production, the impact of such changes on meat quality is unknown.

Nutrition of birds has a significant impact on poultry meat quality and safety. According to Grashorn (2005) the dietary energy supply of birds via carbohydrates or fat directly affects fatness of carcasses. Low-fat, carbohydrate-rich diets do not influence sensory characteristics (Moran, 2001) but decrease carcass fat, carcass yield and breast meat yield (Smith *et al.*, 2002). Hess (2004) reported that feeding diets with a high nutritive density (high energy, high protein) resulted in an improved carcass yield and decreased fatness, with more distinct responses in males. Reducing dietary fat and increasing crude protein or single amino acids increases the contents of protein and amino acids in carcasses (Waldroup *et al.*, 2001). Additional supplementation of lysine improves feed conversion, carcass yield and breast meat yield (Waldroup *et al.*, 2001). It is well-known that dietary fatty acid profiles are reflected in tissue fatty acid profiles. In the past, many papers dealt with enriching poultry meat with n-3 fatty acids by dietary fat sources for improving nutritive value (Crespo & Esteve-Garcia, 2001). It has been suggested that antioxidant balance is responsible for maintaining animal health, productive and reproductive performances of farm animals. In general, an excess of free radicals, or lack of antioxidant protection, can shift this balance producing oxidative stress which can cause losses in the productive performance, as well as losses in both nutritional and organoleptic quality of the products derived from them. Among the improvements in the quality of the meat due to an increase in antioxidant supplementation we could highlight: Improvements in color and oxidative stability of lipids, Reduction of odor and rancid taste, both raw and cooked and an increase of the water retention capacity. Antioxidants in grape pomace prevent the degradation of fatty acid. The anthocyanins are colored water-soluble pigments belonging to the phenolic group and the colored anthocyanin pigments have been traditionally used as a natural food colorant. Therefore, the anthocyannins in GP may influence the color of the meat. In addition to their coloring

efficiency, increasing evidence suggests that anthocyanins are not only non-toxic and mutagenic, but also have a wide range of therapeutic properties.

2.12 Summary

The annual production of grape pomace along with its multitude of applications, create an opportunity to discover an unexploited market with great commercial potential. With increasing consumer demand for the use of less synthetic and more organic compounds, the utilization of by-products (natural compounds) for alternative uses has been a focus of research (Cheng *et al.*, 2010; Rockenbach *et al.*, 2011). Grape pomace has been included in chicken diets with mixed results on feed intake, feed utilization efficiency and growth parameters (Chamorro *et al.*, 2012; Pop *et al.*, 2015).

The use of feed enzymes in poultry production have been adopted to complement endogenous digestive enzymes and this approach is likely to be useful when nonconventional feedstuffs such as GP are being incorporated in chicken diets. The inclusion of these enzymes enhance digestibility, improve gut morphology and improve growth performance of farm animals. Grape pomace contains high level of fibre and polymeric polyphenols as procyanidins with capacity to bind and precipitate both dietary and endogenous proteins and, therefore, the incorporation of GP at high doses in chicken diets might impair nutrient digestion and growth. It is, therefore, imperative that for each broiler strain, the optimum dietary inclusion level of GP be determined so as not to compromise growth performance of the bird. Where higher dietary inclusion levels of GP are desired, it is important to find ways in which the main antinutritional components of GP, fibre and tannins, can be ameliorated. The objective of this thesis is to fill the information gap that exist in this respect.

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3 CHAPTER THREE - GROWTH PERFORMANCE, BLOOD PARAMETERS, AND CARCASS AND MEAT QUALITY CHARACTERISTICS OF COBB 500 BROILER CHICKENS IN RESPONSE TO INCREMENTAL LEVELS OF RED GRAPE POMACE

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3.1 Abstract

The disposal of red grape pomace (GP) in landfills and by incineration has negative impacts on the environment. It is thus imperative that alternative and sustainable ways of managing this waste product are identified. Using GP as a nutraceutical in avian diets is a potential waste-reduction strategy in service of sustainable intensification. This study, therefore, investigated the effect of red grape pomace (GP)-containing chicken diets on growth performance, blood parameters and carcass characteristics on broiler chickens. A feeding trial was conducted using four hundred, two-week old Cobb 500 broiler chickens (279.2 ± 18.87 g) to evaluate their physiological and meat quality traits in response to incremental levels of GP. The chickens were randomly and evenly allocated to a total of 40 pens (experimental units) measuring 3.5 m long × 1.0 m wide × 1.85 m high in a broiler house. The broilers were reared on five isonitrogenous and isoenergetic commercial chicken diets containing graded levels of GP as follows: GP0 = commercial chicken diet without GP; GP25 = commercial chicken diet containing 2.5% GP; GP45 = commercial chicken diet containing 4.5% GP; GP55 = commercial chicken diet containing 5.5% GP; and GP75 = commercial chicken diet containing 7.5% GP. The dietary treatments were thus replicated 8 times with each pen (experimental unit) carrying 10 chickens. The diets were offered *ad libitum* to the birds over a 4-week period. Fresh water was available at all times. Weekly feed intake and body weights were recorded and used to calculate average weight gain (AWG) and feed

conversion ratio (FCR). Blood was collected from brachial vein at 40 days of age for analysis of haematological and serum biochemical parameters. At the end of the 4-week feeding trial, all chickens were slaughtered at a local abattoir to assess carcass and meat quality characteristics. Level of dietary GP inclusion quadratically affected overall feed conversion ratio [$Y = 0.56\ (\pm 0.015) - 0.001\ (\pm 0.0009)x + 0.0004\ (\pm 0.00001)\ x^2$] ($P = 0.003$; $R^2 = 0.557$). There were no linear and quadratic trends on haematology, serum biochemistry, and carcass characteristics of broiler chickens fed incremental levels of GP-containing diets. However, linear trends were observed for breast meat pH, redness and hue angle. The dietary treatments had a significant effect ($P < 0.05$) on average weekly feed intake (AWFI) (g/bird) of broiler chickens. Diet GP55 promoted the least AWFI in week 3 (369 g/bird), while GP75 promoted the least AWFI from week 4 to 6 (387.8, 426.6 and 521.8 g/bird). However, there were no dietary effects on overall weight gain. The commercial diet without grape pomace (GP0) promoted the lowest FCR (2.308) while GP75 had the highest (2.835) in broilers. The dietary treatments had similar effects on carcass characteristics and internal organs of broiler chickens. The dietary treatment had a significant effect ($P < 0.05$) on redness and hue angle of the meat. GP75 had the highest (0.75) redness of the meat meanwhile, GP0 had the least (0.49). In addition, the hue angle decreased as the inclusion level of GP increased with GP0 having the highest (1.54) and GP75 had the least (1.52). However, there were no dietary effects on meat pH, meat temperature and chroma of the meat. Based on feed intake and FCR, it was concluded that 7.5% red grape pomace was the maximum tolerable inclusion rate for Cobb500 broilers. Inclusion of GP in commercial chicken diets beyond this maximum (7.5%) may be necessary to further lower feed costs and promoter greater intake of beneficial bioactive compounds. However, this would require the use of feed enzymes and polyethylene glycol to enhance GP intake and utilization in broilers.

Keywords: blood parameters; broilers; grape pomace; growth performance; meat quality

3.2 Introduction

The poultry industry supports many large and small-scale enterprises and provides a strong platform for rural development, as well as the state food security programme as it is the main supplier of animal protein. The increasing human population as well as greater health concerns around red meat consumption, have led to an increased demand for white meat worldwide. Poultry meat is among the cheapest sources of animal protein and is the most preferred and consumed meat followed by pork and beef worldwide (Le Bihan-Duval, 2004). The production and consumption of poultry meat and products increase over the years worldwide due to the associated desirable nutritional properties, particularly the high protein content, low fat and relatively high levels of polyunsaturated fatty acids (PUFAs) when compared to other meat products (Brenes & Roura, 2010). However, the cost of feeding has emerged as a major constraint to poultry production leading to greater efforts to explore alternative feed ingredients for least-cost and effective poultry production (Wickramasuriya, 2015). Grape (*Vitis vinifera L.*) is said to be one of the largest grown fruit crop in the world, with an approximate annual production of 61 million metric tons (Dorri *et al.*, 2012). Grape pomace (GP) is a wine by-product consisting of peels (skins), seeds and stems particularly rich in a wide range of polyphenols and other phytochemical compounds. The continuous use of antibiotics has been detected in broiler production with this reason a need to use natural bioactive compounds, such as those in GP to improve the physiological response of broilers. Grape pomace is usually neglected and treated as a waste product with no efficient utilisation and the importance of the bioactive compounds in the GP is overlooked.

The phytochemicals present in GP have antioxidant, antimicrobial and health-promoting effects in different biological and food systems (Aditya, 2018). Indeed, GP polyphenols have been shown to reduce toxicity caused by free radicals and prevent oxidative damage of biological macromolecules. Furthermore, bioactive compounds are known to improve feed

utilisation and meat quality attributes. According to Goni *et al.* (2007) the polyphenols contribute significantly to defence of animal organisms by increasing the levels of endogenous antioxidant molecules and enzymes such as glutathione (GSH) and catalase (CAT), and consequently enhancing their immune system. Thus, GP constitutes an inexpensive source of antioxidant polyphenols, which can be used as dietary supplements or in the production of bio functional foods (Alonso *et al.*, 2002). High polyphenol content and high fibre fraction are the major limitations of using GP in broiler diet. It is important to determine the maximum inclusion level of GP in broiler diets given the wide variation in the antinutritional effects of condensed tannins and fibre. Therefore, this study was designed to determine the growth performance, haemato-biochemical parameters, and carcass and meat quality traits of broilers fed diets containing incremental levels of red grape pomace.

3.3 Materials and methods

3.3.1 Ethics statement

The procedures used to rear and slaughter broiler chickens were reviewed and approved by the Animal Research Ethics Committee, North West University (approval no. NWU-00239-18-A5).

3.3.2 Description of the study site

The feeding trial was conducted at the North-West University Research Farm (Molelwane) with geographical coordinates of 25°40.459' S, 26°10.563' E, in the North West province of South Africa. The feeding trial was done in winter (May- June) and temperatures during this time range from -3°C to 25°C.

The feeding trial was conducted in a broiler house with 40 pens measuring 3.5 m long × 1.0 m wide × 1.85 m high were used. Each dietary treatment was replicated 8 times with 10

chickens per pen. The temperatures in the house were monitored through a thermometer and regulated by rolling up or down the curtains of the broiler house.

3.3.3 Diet formulation

Fresh red grape (*Vitis vinifera L. var. Shiraz*) pomace was supplied by Blaauwklippen Wine Estate, located at 33, 9692° S, 18, 8444° E (Stellenbosch, South Africa). The climatic conditions in the Stellenbosch are Mediterranean, with cold, wet winters and dry hot summers, the average temperature is 16.4°C and the average annual rainfall is 802 mm and the soils range from dark alluvium to clay. The diets were formulated by a commercial feed manufacturing company, Nutroteq SA while the broiler chickens were supplied by a farmer in Pretoria, SA. Five experimental diets were formulated to meet the daily nutritional requirements of growing and finishing chickens according to NRC (1994) guidelines. The isonitrogenous and isoenergetic experimental diets were formulated to contain graded levels of GP as follows: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP as shown in Table 3.1.

Table 3.1. Ingredient composition (g/kg as fed) of grape pomace-containing diets

	[1]Dietary treatments									
	Grower					Finisher				
Ingredients	GP0	GP25	GP45	GP55	GP75	GP0	GP25	GP45	GP55	GP75
Grape pomace	0	25	45	55	75	0	25	45	55	75
Soy oilcake	245	197	149	124	74	168	110	75	40	0
Full fat soya	10	10	55	86	150	55	125	168	210	259
Gluten 60	5	23	33	34	35	0	0	0	0	0
Lysine	1.39	2.54	2.79	2.78	2.75	1.93	1.85	1.8	1.75	1.67
Methionine	1.42	1.29	1.11	1.05	0.94	1.51	1.38	1.3	1.22	1.12
Threonine	0	0.08	0.07	0.06	0.04	0.1	0.06	0.04	0.03	0
Yellow maize	709	712	686	668	633	751	714	692	670	640
Feed lime	14.6	14.3	14	13.8	13.4	12.5	12.2	12.1	11.9	11.7
Monocalcium phosphate	7	7.4	7.6	7.6	7.7	2.2	2.3	2.3	2.3	2.3
Salt-fine	3.29	3.25	3.2	3.23	3.28	2.78	2.86	2.9	2.95	3.02
Sodium bicarbonate	1.6	1.6	1.6	1.6	1.6	1.9	1.9	1.7	1.6	1.6
Axtra phy	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline	0.8	0.8	0.8	0.8	0.8	0	0	0	0	0
Salinomycin	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0
Olaquindox	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2
Premix	0.5	0.5	0.5	0.5	0.5	2.5	2.5	2.5	2.5	2.5
Zinc bacitracin	0	0	0	0	0	0.5	0.5	0.5	0.5	0.5
Soy crude oil	0	0	0	0	0	0	0	0	0	1.3

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

3.3.4 Chemical analysis

Red grape pomace and the formulated diets were sampled and dried in an oven set at a temperature of 60°C until constant weight and then milled to pass through a 1 mm sieve for preliminary chemical analyses. For laboratory dry matter (DM) determination, approximately 1 g of sample was placed into pre-weighed crucibles and placed in an oven set at 105°C for 12 hours. The loss in weight was measured as moisture content and DM was calculated as the difference between the initial sample and moisture weights. Organic matter content (OM) was determined by ashing the dried samples in a muffle furnace set at 600°C for 12 hours. The loss in weight was measured as organic matter (OM) content and the residue as ash. Total nitrogen content was determined by the standard macro-Kjeldahl method (AOAC, 1999: method no. 984.13) and was converted to crude protein by multiplying the percentage N content by a factor of 6.25 and expressed in g/kg DM. Crude fibre was determined using the ANKOM2000 Fibre analyser (ANKOM Technology, New York) with 0.255 N crude fibre acid solution and then with 0.313 N crude fibre base solution. The energy content was determined using a bomb calorimeter and measured as kilocalories (kcal). Soluble phenolics and total polyphenolics: estimated with Folin-Ciocalteau method (Makkar, 2003). Soluble condensed tannins were assayed using the Butanol-HCl method (Porter *et al.*, 1986).

Mineral content (calcium, phosphorus, sodium, chloride and potassium were analysed in the Animal Health laboratory using the dry ashing macro and trace minerals methods, following the gui0delines provided by the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). Samples that were used to determine the DM were further incinerated in a muffle furnace for 12 h. The ash was weighed and digested with 1 mL of 55% nitric acid and 10 mL of 32% hydrochloric acid using a Microwave Reaction System Model 3000. Samples were digested for 45 minutes, cooled, and transferred into respective volumetric flasks (100 mL),

which was eventually topped-up with distilled water and left standing for 24 h to allow the sediment to settle down. After 24 h, samples were slowly transferred to McCartney bottles without disturbing the sediment. The mineral concentrations were determined using an ICP Mass Spectrometer (Perkin-Elmer, NexION 300Q).

3.3.5 Experimental design

A total of four hundred, day old Cobb500 broiler chickens were obtained from a farm in Pretoria (Gauteng, SA). The chickens were randomly and evenly allocated to a total of 40 pens in a broiler unit (experimental units) and thus the dietary treatments were replicated 8 times with each pen carrying 10 chickens.

The study was arranged in a completely randomized design. The pens (measuring 3.5 × 1.0 × 1.85 m) were designed to meet the animal welfare standards for optimum production of chickens. The day-old broiler chicks were fed the commercial starter from Nutri-feeds until 10 days and were then adapted to the experimental diets formulated by Nutroteq (PTY) LTD for 3 days and measurements commenced on day 14 to 42.

3.3.6 Feeding and broiler management

Experimental diets were formulated according to the commercial feed formulation standards to meet the nutrient requirements for the grower and finisher phases. Dietary treatments and fresh water were provided *ad libitum* and average daily feed intake was measured from week 3 to week 6. Feed intake was measured daily and live weight was measured weekly. All birds from the forty pens were weighed at the beginning of the trial (initial body weight) and subsequently weighed weekly (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA). The feed offered was weighed before

feeding and refusals were collected each morning before feeding and weighed. Feed conversion ratio was determined as a proportion of feed intake to weight gain.

3.3.7 Blood collection and analysis

At 40 days of age 2 chickens were randomly chosen from each pen for blood collection; blood was collected from the brachial vein using needle and syringe. Purple-top tubes containing ethylene diamine tetra acetic acid as an anti-coagulant were used to collect blood for haematological analyses while red-top tubes without anticoagulant were used to store blood for serum biochemical analysis. The blood samples were analysed using the IDEXX Catalyst one chemistry analyzer and IDEXX Laser Cyte Dx Haematology analyser equipment in North-West University Animal Science laboratory (Mafikeng, SA). The Idexx Lasercyte (Haematology analyser) was used to analyse for haematocrit, haemoglobin, erythrocyte, leucocyte, neutrophils, lymphocytes, monocytes, eosinophil and normoblasts. Total protein (TP), albumin, cholesterol and mineral content were the serum biochemical components that were analysed following guidelines by Buetow *et al.* (1999).

3.3.8 Slaughter procedures

At 6 weeks of age, all broiler chickens were starved for 24 hours and taken to Rooigrond poultry abattoir (North West province, South Africa) for slaughter. At the abattoir, all the chickens were stunned and live-hanged onto a movable metal rack that holds them upside down by their feet. Chickens were then slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding stopped. The chickens were then defeathered and the carcases were taken to the NWU Meat Science Laboratory for measurements.

3.3.9 Carcass traits and internal organs

After slaughter, carcasses from the different dietary treatments and replicate pens were placed in labelled plastic bags. Thereafter, carcasses were eviscerated and carcass traits measured. The following were removed and weighed: gizzards, livers, proventriculi, breasts, thighs, drumsticks and wings. Length of small intestines and large intestines were also measured and recorded. Hot carcass weights (HCW) were measured before the carcasses were chilled for 24 h to acquire the cold carcass weight (CCW). The carcass weight of each chicken was recorded and dressing out percentage was calculated. Breast (pectoralis major muscle) samples were carefully removed after 24 hours of slaughter for evaluation of meat quality traits. Breast samples were then vacuum packed and kept frozen (−20°C) pending meat quality analysis at NWU laboratory.

3.3.10 Meat quality measurements

3.3.10.1 Meat pH and temperature

Meat pH and temperature were recorded immediately after slaughter and 24 h post slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford *et al.* (2003). After every 20 measurements, the pH meter was calibrated with pH 4, pH 7 and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) at a temperature of 2 °C.

3.3.10.2 Meat colour

Colour of the meat (L^* = Lightness, a^* = Redness and b^* = Yellowness) was determined, immediately after slaughter(HC) and also 24 hours after slaughter(CC), using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany), on a 20 mm diameter

measurement area and illuminant D65-day light, 10° observation angle. The colour meter was calibrated using the green standard before measurements. Colour recording was done on the surface of the thigh muscle, which was allowed to bloom for 1 hour on a polystyrene tray at 4 °C. Hue angle was calculated as $\tan(\theta) = \frac{a^*}{b^*}$, and chroma was calculated as $\sqrt{a^{*2} + b^{*2}}$ as guided by Priolo *et al.* (2002).

3.3.10.3 Water holding capacity

The water holding capacity (WHC) of the meat was measured in duplicate samples on the surface of a freshly cut slice of the pectoralis major muscle (PMM) (8-16 grams). The WHC was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau and Hamm (1957). The water from the fresh meat was taken up by a pre-weighed filter paper and calculated as a percentage. Water holding capacity was calculated using the equation:

$$WHC\ (\%) = \frac{Initial\ weight - Weight\ after\ pressing}{Initial\ weight} \times 100$$

3.3.10.4 Drip loss

Drip loss was determined using a method adapted from Zhang *et al.* (2009). Pieces of muscle from the pectoralis major muscle (PPM) weighing ~ 2 grams (wet weight, w1) were hooked using a wire and suspended in a plastic container so that the samples did not touch the sides of the bottle, which was then sealed. The suspended samples were stored in a cold room at 4°C for 72 hours. The meat samples were then reweighed to obtain weight after drip (w2).

The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$Drip\ loss\ (\%) = \frac{w1 - w2}{w1} \times 100,$$

where *w1* is initial weight and *w2* is weight after drip.

3.3.10.5 Cooking loss

Raw breast muscle samples chilled overnight at 4°C chiller were individually weighed to obtain initial weight (*w1*) after thawing. The samples were then placed in foil plate and oven broiled (dry heating) at 180°C for 30 minutes. The broiled samples were then removed from the oven and left to cool for 20 minutes. The samples were then re-weighed to obtain the cooked weight (*w2*) of the PMM). The cooking loss was calculated based on the difference between the weight of raw meat and cooked meat using the following equation:

$$Cooking\ loss\ (\%) = \frac{w1 - w2}{w1} \times 100,$$

where *w1* is the weight of raw meat and *w2* is weight after cooking.

3.3.10.6 Meat tenderness

The breast muscle samples that were previously cooked at 180°C for 30 minutes and used for determination of cooking loss were then used for the shear force evaluation. The subsamples of 2 cm high × 2 cm width × 12 cm length dimension were sheared perpendicular to the fibre direction using a Meullenet - Owens Razor Shear Blade (A/MORS) mounted on a Texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value in Newtons (N) represented the average of the peak force measurements of each sample.

3.3.11 Statistical analysis

Data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Proc RSREG; SAS 2010) was applied to describe responses of parameters to graded levels of grape pomace in diets fed to Cobb 500 broiler chickens, according to the following quadratic model: $y = ax^2 + bx + c$, where y = response variables, a and b are the coefficients of the quadratic equation; c is intercept; x is dietary GP level (%). Weekly feed intake, weight gain and FCR data were analysed using the repeated measures analysis SAS (2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

where, Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed.

Overall feed intake, weight gain, FCR, blood parameters, carcass characteristics and meat quality data were analysed using the general linear model procedure of SAS (2010). The linear statistical model employed was as follows:

$$Y_{ik} = \mu + D_i + E_{ik}$$

Where, Y_{ik} = dependant variable, μ = population mean, D_i = effect of diets and E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

3.4 Results

The commercial grower diet treated with 7.5% of grape pomace had the highest fibre content, while sodium content remained the same across all the dietary treatments (Table 3.2).

Table 3.2. Chemical composition (g/kg, unless otherwise stated) of grape pomace-containing diets

	[1]Dietary treatments									
	Grower					Finisher				
[2]Parameters	GP0	GP25	GP45	GP55	GP75	GP0	GP25	GP45	GP55	GP75
Dry matter	893.7	895.0	897.7	899.3	902.6	888.6	892.9	895.5	898.1	901.4
ME (MJ/kg)	119.0	118.9	119.0	119.0	119.02	122.0	121.9	121.9	122.0	121.9
Protein	170.0	170.0	170.1	170.0	169.75	160.0	160.0	160.0	160.0	160.0
Fat	33.53	34.31	42.23	47.66	58.87	42.66	54.16	61.19	68.10	77.48
Fibre	25.0	35.6	45.4	50.7	61.4	35.2	46.7	53.7	60.6	69.7
OM	844.1	846.9	849.7	851.1	853.9	848.9	852.7	854.9	857.3	860.1
Calcium	8.21	8.19	8.20	8.19	8.19	6.59	6.60	6.62	6.61	6.62
Phosphorus	4.99	4.91	4.86	4.83	4.77	3.41	3.40	3.38	3.35	3.33
Sodium	1.80	1.80	1.80	1.80	1.80	1.60	1.60	1.60	1.60	1.60
Chloride	2.81	3.00	3.00	3.00	3.00	2.50	2.50	2.50	2.50	2.50
Potassium	7.52	6.95	6.91	6.99	7.18	6.55	6.75	6.87	6.98	7.15

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP; [2]Parameters: ME = metabolisable energy; OM = organic matter.

There were significant quadratic trends for feed intake on week 3 [Y = 454.3 (±7.158) – 2.58 (± 0.416)x + 0.0195 (± 0.005)x^2], week 5 [Y = 568.2 (±14.321) + 1.713 (± 0.832)x – 0.047 (± 0.011)x^2] and week 6 [Y = 699.9 (±19.942) + 0.702 (± 1.160)x – 0.042 (± 0.015)x^2] however, a linear effect [Y = 507.9 (±10.130) – 0.63 (± 0.59)x] was observed for feed intake in week 4. There were no significant linear and quadratic trends for weight gain. The FCR linearly increased in week 3 [Y = 0.726 (±0.019) + 0.002 (± 0.002)x] and week 4 [Y = 0.481 (±0.037) – 0.05 (±0.002)x]. However, quadratic effects were observed in week 5 [Y = 0.481 (±0.030) – 0.05 (± 0.002)x + 0.00002 (± 0.00003)x^2] and no linear and quadratic trends for week 6. The repeated measures analysis showed no significant (P >0.05) effect on overall weight gain and overall feed conversion ratio, however, was significant on average weekly feed intake, with GP55 promoting the least AWFI in week 3 (369.6 g/bird) and GP75 promoting the least AWFI from week 4 to 6.

Table 3.3. Average weekly feed intake (g/bird) in broiler chickens fed diets containing grape pomace

	[1]Dietary treatments					[2]Significance	
	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Week 3	454.8^{b}	396.3^{a}	381.3^{a}	369.6^{a}	370.3^{a}	***	***
Week 4	505.9^{d}	484.4^{cd}	456.1^{bc}	421.7^{ab}	387.8^{a}	***	NS
Week 5	574.9^{c}	554.2^{bc}	581.1^{c}	504.8^{b}	426.6^{a}	***	***
Week 6	702.0^{b}	672.6^{b}	694.4^{b}	564.7^{a}	521.8^{a}	***	**

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Significance: NS = not significant; *** = $P < 0.001$, ** = $P < 0.01$.

[a,b] In a row, dietary treatment means with common superscripts do not differ ($P < 0.05$).

There were significant quadratic trends for overall feed intake [Y = 2230.39 (±45.553) – 0.0802 (± 2.649)x – 0.084 (± 0.034)x^2] and overall FCR [Y = 0.56 (±0.015) – 0.001 (± 0.0009)x + 0.00004 (± 0.00001)x^2] but not for overall weight gain in response to incremental levels of dietary GP. The effect of grape pomace containing diets on overall weight gain and overall feed conversion ratio (FCR) are presented in Table 3.4. There was a significant ($P < 0.05$) difference between the diets in terms of overall FCR but not ($P > 0.05$) in terms of overall weight gain. The overall FCR of the birds ranged from 2.308 – 2.835. The birds fed GP75 had the highest ($P < 0.05$) overall FCR (2.835) while the birds fed the control diet (GP0) had the least overall FCR (2.308).

Table 3.4. The effect of grape pomace-containing diets on overall feed intake, weight gain and feed conversion ratio.

	[1]Dietary treatments					[4]Significance	
	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
[2]Overall WG (g)	1253.2	1205.9	1204.8	1172.1	1176.3	NS	NS
[3]Overall FCR	2.308[a]	2.379[a]	2.427[ab]	2.632[bc]	2.835[c]	***	*

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Overall WG = overall weight gain.

[3]Overall FCR = overall feed conversion ratio.

[4]Significance: NS = not significant; *** = $P < 0.001$.

[a,b]In a row, dietary treatment means with common superscripts do not differ ($P > 0.05$).

Table 3.5. The haematological parameters of broiler chickens fed grape pomace-containing diets

	[1]Dietary treatments					[3]Significance	
[2]Parameters	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Erythrocyte($\times10^{12}$/L)	1.58	1.28	1.47	1.41	1.29	NS	NS
Haematocrit (L/L)	11.30	12.55	11.61	11.69	11.78	NS	NS
MCV (fl)	54.19	56.76	60.05	62.14	57.40	NS	NS
MCH (pg)	45.68	41.15	48.58	53.79	44.01	NS	NS
RDW ($\times10^{9}$/L)	37.29	35.98	33.03	31.99	33.91	NS	NS
Haemoglobin (g/dl)	9.41	9.20	9.33	9.56	8.93	NS	NS
NEU ($\times10^{9}$/L)	7.53	8.42	6.82	7.75	13.17	NS	NS
LYMP ($\times10^{9}$/L)	22.43	11.78	11.55	19.43	16.42	NS	NS
MONO ($\times10^{9}$/L)	21.97	10.58	9.36	20.62	12.05	NS	NS
EOS ($\times10^{9}$/L)	1.18	1.06	1.05	1.00	1.36	NS	NS
RETIC (K/μL)	314.5	335.1	293.8	211.2	166.1	NS	NS
WBC ($\times10^{9}$/L)	52.80	30.79	28.93	48.97	43.12	NS	NS
BASO ($\times10^{9}$/L)	0.16	0.17	0.15	0.17	0.13	NS	NS

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Parameters: MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; RDW=red blood cell; NEU = Neutrophils; LYMP = lymphocytes; MONO = monocytes; EOSO = eosinophils; RETIC = reticulolyte; WBC = white blood cell; BASO = basophils.

[3]Significance: NS = not significant

There were neither linear nor quadratic trends on haematological parameters of broiler chickens in response to incremental levels of dietary GP. For haematological parameters, Table 3.5 shows that dietary treatments had no significant effect ($P > 0.05$) on erythrocyte count, haematocrit, MCV, MCH, RDW, haemoglobin, NEU, LYMP, MONO, EOSO, RETIC, WBC, BASO of broiler chickens.

Table 3.6. Effect of GP-containing diets on serum biochemical parameters of broiler chickens

	[1]Dietary treatments					[3]Significance	
[2]Parameters	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Glucose (mmol/L)	17.31	17.07	17.25	19.59	17.72	NS	NS
Calcium (mmol/L)	3.34	3.48	3.20	3.46	3.18	NS	NS
Creatinine (µmol/L)	9.64	9.57	9.67	9.71	9.00	NS	NS
Total protein (g/L)	46.50	49.25	45.33	46.93	45.83	NS	NS
Albumin (g/L)	0.64	0.69	0.68	0.64	0.66	NS	NS
Phosphorus (mmol/L)	2.66	2.93	2.77	2.71	2.64	NS	NS
Globulin (g/L)	30.13	29.44	28.42	31.25	27.83	NS	NS
Total bilirubin (µmol/L)	9.69	8.94	7.75	10.25	10.25	NS	NS
ALT (U/L)	30.69	27.69	19.75	30.88	35.36	NS	*
ALKP (U/L)	754.6	805.9	731.4	695.0	727.2	NS	NS
GGT (U/L)	31.64	29.06	31.33	34.44	24.00	NS	NS
Cholesterol (mmol/L)	5.12	5.28	4.62	4.90	4.76	NS	NS
Amylase (U/L)	433.6	321.3	356.5	356.5	321.3	*	NS
Lipase (U/L)	135.3	142.4	142.8	161.8	144.3	NS	NS

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Parameters: ALT=Alanine transaminase; ALKP = Alkaline phosphate; GGT = Gamma glutamyl transferase.

[3]Significance: NS = not significant.

There were neither linear nor quadratic trends for serum biochemical parameters except for ALT [Y = 33.41 (±5.697) – 0.839 (±0.331)x + 0.012 (±0.004)x^2] and amylase [Y = 424.85 (±48.393) – 3.44 (±2.814)x]. Table 3.6 shows that there were no dietary influence ($P > 0.05$) on biochemical parameters of broiler chickens fed commercial diets containing GP. Glucose ranged from 17.07 to 19.59 mmol/L, whereas albumin ranged from 0.64 to 0.69 g/L. Cholesterol ranged from 4.62 to 5.28 mmol/L. There were no significant linear and quadratic trends for internal organs with the exception of proventriculi [Y = 0.502 (±0.026) + 0.0007 (±0.002)x]. The effect of experimental diets on relative size of internal organs (% HCW) of broilers is presented in Table 3.7. There was no dietary influence ($P > 0.05$) on size of internal organs of broiler chickens.

Table 3.7. The effect of grape pomace-containing diets on relative size of internal organs (% HCW) of broilers

	[1]Dietary treatments					[2]Significance	
Organs	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Gizzards	3.03	3.17	3.28	3.13	3.34	NS	NS
Proventriculi	0.49	0.57	0.55	0.54	0.56	*	NS
Livers	3.23	3.12	2.97	3.60	3.12	NS	NS
Small intestines	150.4	153.0	152.8	148.6	148.4	NS	NS
Large intestines	9.78	8.45	9.90	9.39	9.69	NS	NS

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Significance: NS = not significant.

For carcass characteristics, there were no significant linear and quadratic trends on dressing percentage, cooking loss, wings, drumstick and thighs, however, slaughter weight (SW) and hot carcass weight (HCW) decreased linearly while quadratic effects were observed for breast weight [$Y = 7.69\ (\pm 1.176) + 0.701\ (\pm 0.068)x - 0.007\ (\pm 0.001)x^2$]. Table 3.8 indicates that the diets had no significant ($P > 0.05$) effect on carcass characteristics lengths (cm) of broiler chickens.

Table 3.8. The effect of red grape pomace-containing diets on carcass traits of broiler chickens

Parameters	[1]Dietary treatments					[5]Significance	
	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Dressing %	69.64	70.98	72.43	71.31	70.94	NS	NS
Breast (% HCW)	21.39	21.92	27.60	23.09	17.08	***	***
Wing (% HCW)	5.84	6.22	5.87	5.99	6.25	NS	NS
Drumstick (% HCW)	6.48	6.97	6.85	6.80	6.65	NS	NS
Thigh (% HCW)	8.01	8.61	8.11	7.94	6.68	NS	NS
[2]HCW (% HCW)	1299.6	1218.9	1256.5	1184.6	1155.6	*	NS
[3]CCW (% HCW)	1270.7	1229.2	1237.1	1161.9	1153.1	NS	NS
[4]SW (% HCW)	1812.9	1720.8	1736.8	1660.6	1627.8	*	NS
Wing (cm)	19.34	17.32	17.96	17.28	18.16	NS	NS
Drumstick (cm)	11.34	10.74	10.92	10.56	11.00	NS	NS
Thigh (cm)	9.53	8.89	8.72	9.44	8.77	NS	NS
Back (cm)	18.89	18.47	18.92	29.66	18.86	NS	NS

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]HCW = hot carcass weight; [3]CCW = cold carcass weight; [4]SW = slaughter weight; [5]Significance: NS = not significant; * = $P < 0.05$

No linear and quadratic trends were observed for temperature, lightness (L*), yellowness (b*) and chroma of the breast meat, however, linear trends were observed for meat pH [Y = 6.45 (±0.059) + 0.0007 (± 0.003)x], redness (a*) [Y = 0.518 (±0.022) + 0.002 (± 0.0013)x] and hue angle [Y = 1.539 (±0.002) – 0.0001(± 0.00009)x]. Table 3.9 shows the meat quality parameters of broiler chickens fed diets containing incremental levels of red grape pomace. The dietary treatments had no effect ($P > 0.05$) on pH, temperature, lightness, yellowness and chroma of the breast meat. However, a significant ($P < 0.05$) effect was observed for redness and hue angle of the meat. Meat from broiler chickens fed the control diet (GP0) had the least redness value (0.49) while meat from GP75 chickens had the highest redness value (0.75). Hue angle ranged from 1.52 (GP75) – 1.54 (GP0).

Table 3.9. The effect of red grape pomace-containing diets on meat quality parameters of broiler chickens

	[1]Dietary treatments					[2]Significance	
	GP0	GP25	GP45	GP55	GP75	Linear	Quadratic
Meat pH	6.37	6.31	6.31	6.41	6.36	*	NS
Temperature (°C)	25.90	26.56	26.47	26.30	26.38	NS	NS
*L**	55.92	57.31	56.34	55.53	56.32	NS	NS
*a**	0.49^{a}	0.62^{b}	0.62^{b}	0.66^{b}	0.75^{c}	*	NS
*b**	16.34	15.94	16.75	16.20	16.11	NS	NS
Chroma	16.35	15.95	16.77	16.21	16.13	NS	NS
Hue angle	1.54^{c}	1.53^{bc}	1.53^{abc}	1.53^{ab}	1.52^{a}	*	NS

[1]Dietary treatments: GP0 = Commercial chicken diet without GP; GP25 = Commercial chicken diet containing 2.5% GP; GP45 = Commercial chicken diet containing 4.5% GP; GP55 = Commercial chicken diet containing 5.5% GP; and GP75 = Commercial chicken diet containing 7.5% GP.

[2]Significance: NS = not significant; *** = $P < 0.001$.

[a,b,c] In a row, dietary treatment means with common superscripts do not differ ($P > 0.05$).

Figure 3.1 below shows that dietary treatments had no effect ($P > 0.05$) on water holding capacity (3.33 – 5.99%), shear force (5.66 – 6.81 N) and drip loss (16.93 – 18.93%) but affected ($P < 0.05$) cooking losses (18.16 – 26.13%). Dietary treatment GP45 promoted higher cooking losses than diets GP0, GP25, GP55 and GP75, which did not differ ($P > 0.05$).

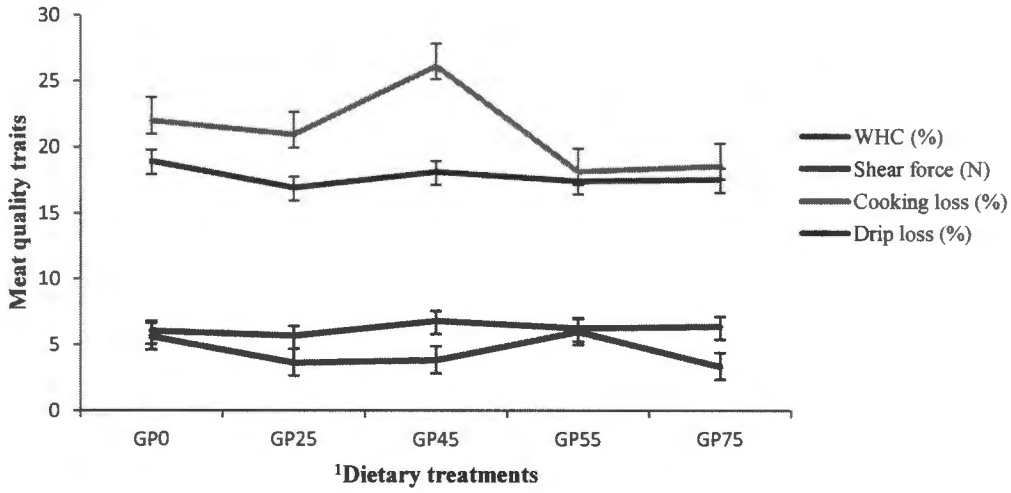

Figure 3.1. Water holding capacity (WHC), shear force, cooking loss and drip loss of meat from broiler chickens reared on red grape pomace-containing diets.

[[1]Dietary treatments: GP0 = commercial broiler diet without grape pomace, GP25 = commercial broiler diet containing 2.5% grape pomace, GP45 = commercial broiler diet containing 4.5% grape pomace, GP55 = commercial broiler diet containing 5.5% grape pomace and GP75 = commercial broiler diet containing 7.5% grape pomace]

3.5 Discussion

Determination of growth performance, haematology, serum biochemistry and meat quality parameters of broilers fed commercial broiler diets containing red grape pomace is essential in order to evaluate the utility of this wine-making by-product in optimising the broiler chicken's performance, health and product quality. Red grape pomace has the potential for use as a functional feed in animal nutrition (Brenes *et al.*, 2008) because of high levels of bioactive compounds with beneficial effects as antioxidants and antimicrobials. Yet this by-product also contains anti-nutrients such as fibre and low molecular weight phenolic

compounds. In light of this, it is imperative to identify the maximum tolerance level of red grape pomace in broiler chickens in order to optimize nutrient utilization, growth performance, health and meat quality.

Results from this study show that the highest inclusion level of red grape pomace (7.5%) depressed overall feed intake, a possible result of high amounts of fibre in that diet compared to the rest of the diets. This finding corroborates the results by Singh *et al.* (2017) where higher levels of fiber in broiler diets reduced feed utilization. In addition, Lau and King (2003) reported that broiler chicks fed diets with grape pomace seed extract at different levels decreased feed intake as the level of the extract increased. Initially the dilution of the energy density of the diet with fibre actually increases feed intake as the chicken eats more to compensate for low energy density. However, as the fibre level continues to increase, the capacity of the stomach becomes limiting because the feed is digested slowly since the chickens do not produce cellulolytic enzymes required to quickly breakdown fibre. Owusu-Asiedu *et al.* (2006) concluded that the amount and type of fibre in the diet affects the gastrointestinal (GIT) development and growth performance in broilers.

Despite the significant variation in feed intake in response to incremental levels of GP, there were neither linear nor quadratic dietary influences on overall weight gain of broiler chickens. These findings are similar to those of Aditya *et al.* (2018) who demonstrated that inclusion of grape pomace at different levels did not significantly affect body weight gain in broilers. Brenes *et al.* (2008) also concluded that the addition of grape pomace concentrate up to 60 g/kg in broiler chicken diets did not change growth performance and organ size. The lack of dietary effects on the overall weight gain for diets suggest that grape pomace inclusion level did not cause any significant changes in physico-chemical properties of the diets. It could also be evidence of the GP's growth-boosting properties such that even though the intake was depressed at higher inclusion levels, this did not result in reduced nutrient

availability to the chicken and thus low feed intake was not accompanied by low weight gain. This further suggests that the broilers at the grower stage have a fully developed digestive system to cope with the higher fibre and secondary plant metabolites observed in the diets except at the highest inclusion level. High polyphenol content and high levels of fibre fraction are the major limitations of using GP in broiler production. Diets containing red grape pomace had an effect on FCR, a result that is in contrast with a report by Kara and Kocaoglu-Guclu (2012) suggesting that inclusion of red grape pomace at 2% has no effect on FCR of moulted laying hens. However, the current findings are in agreement with Pop *et al.* (2015) who concluded that the broiler chickens offered feed containing red grape pomace had better FCR than those offered the control diet.

Haematological and serum biochemical parameters were not influenced by GP containing diets and were within the normal range, in agreement with Pascariu *et al.* (2017). Similar results have been reported by Kara *et al.* (2016) who reported that hens fed GP at 40 and 60 g/kg had similar levels of serum triglycerides as those on the control diet. In addition, Ebrahimzadeh *et al.* (2018) reported no variations in serum biochemical parameters (total protein, glucose and cholesterol) of chicks fed grape pomace. In contrast with the current study, Hajati *et al.* (2015) concluded that grape seed extract supplementation (150, 300 and 450 mg/kg) decreases the concentration of serum glucose.

Diet, genetics, sex, slaughtering conditions and age of the animal are some of the factors that are known to influence carcass traits (Young *et al.*, 2001) in birds. However, in this study, carcass characteristics and size of internal organs were not affected by diets. Similar results have been reported by Aditya *et al.* (2018), Goni *et al.* (2007) and Brenes *et al.* (2008), who reported that the inclusion of graded concentrations of grape pomace concentrate did not affect the size of the liver, pancreas and spleen. On the contrary, Kara (2015) reported that including grape pomace (Dimrit grapes) into diets of laying hens at 4 and 6% increased liver

weight. When diets are highly fibrous, gizzard size is expected to increase as an adaptation mechanism to enhance digestion. Kara *et al.* (2015) reported that including grape pomace into laying hen diets at 4% and 6% increased the liver weight and liver weight ratio, thus providing evidence that the liver grows in size in response to higher levels of toxins. Theoretically, the length of intestines is expected to be longer in chickens fed high levels of fibre. There was no dietary effect on the pH, temperature and lightness of breast meat in agreement with the findings reported by Aditya *et al.* (2018) when investigating the supplementation of grape pomace in broiler diets. Furthermore, a study by Carpenter *et al.* (2007) showed that the addition of grape pomace seed extract on raw pork patties did not change lightness. Carpenter *et al.* (2007) also reported that meat redness did not differ when grape pomace seed extract was added to raw pork patties. This finding is in contrast with the results of this study, which show that feeding chickens diets containing red grape pomace affected the redness and hue angle of the meat. The redness of the meat increased as the inclusion levels of grape pomace increased while the hue angle decreased with the increasing inclusion levels of grape pomace. These results were expected in this study as the anthocyanin and free radicals in GP are known to improve the color and quality of the meat.

3.6 Conclusion

It was concluded that precautions need to be taken when high amounts of grape pomace are included in broiler diets, considering that the diet containing 7.5% red grape pomace promoted the least overall feed intake. This could have been caused by the high level of fibre as well as antinutritional compounds such as condensed tannins found in red grape pomace. Treatment of red grape pomace with polyethylene glycol and feed enzymes may reduce the level of antinutrients and thus allow its inclusion at levels higher than 7.5%. Inclusion of GP in commercial chicken diets beyond this maximum (7.5%) may be necessary to further lower

feed costs and promote greater intake of beneficial bioactive compounds. Further research is required to find ways to improve the feed intake of GP by broilers, especially when included in diets at high levels. This could be achieved by pre-treating the pomace with fibrolytic enzymes and polyethylene glycol to ameliorate the antinutritional effects of fibre and condensed tannins, respectively.

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4 CHAPTER FOUR - EFFECT OF POLYETHYLENE GLYCOL AND FIBROLYTIC ENZYME-TREATED DIETARY RED GRAPE POMACE ON PHYSIOLOGICAL AND MEAT QUALITY PARAMETERS OF BROILER CHICKENS

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4.1 Abstract

The utility of red grape pomace (GP) as a nutraceutical for broilers is limited by anti-nutritional compounds, fibre and condensed tannins. Strategies to ameliorate the anti-nutritional effects of these major components of GP need to be identified and evaluated. The current study, therefore, evaluates the effect of pre-treating GP with polyethylene glycol (PEG) and Viscozyme® (a cellulolytic mixture of arabinase, cellulase, β-glucanase, hemicellulase and xylanase enzymes) on growth performance, carcass characteristics and meat quality parameters of Cobb 500 broiler chickens. A total of 400, two-week old Cobb 500 broiler chickens were randomly and evenly allocated to a total of 40 pens (experimental units) measuring 3.5 m long × 1.0 m wide × 1.85 m high in a broiler unit. Five isoenergetic and isonitrogenous diets were formulated as follows: 1. Commercial chicken diet without red grape pomace (CON); 2. Commercial chicken diet containing 10% red grape pomace (GP); 3. Commercial chicken diet containing 10% red grape pomace pre-treated with polyethylene glycol (5% w/w) (PEG); 4. Commercial chicken diet containing 10% red grape pomace pre-treated with Viscozyme® - L (0.1% w/w) (ENZ); and 5. Commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w) (PENZ). The diets were randomly allocated to the pens and thus were replicated 8 times with each pen carrying 10 chickens. Blood was collected from brachial vein at 40 days of age for analysis of haematological and serum biochemical parameters. At the end of the 4-week

feeding trial, all chickens were slaughtered at a local abattoir to assess carcass and meat quality characteristics. Repeated measures analysis showed that there were no (P >0.05) week × diet interaction effects on average weekly FI, average WG and FCR. There were also no dietary influences (P >0.05) on overall feed intake (g/bird) and overall FCR. The CON diet promoted the highest overall WG (1351.4 g/bird), which did not differ (P >0.05) from PEG, ENZ and PENZ diets, while the least overall WG (1188.9 g/bird) was observed in chickens fed on GP diet. There were significant dietary effects on slaughter weight, hot carcass weight (HCW), cold carcass weight (CCW) and water holding capacity (WHC) of broiler chickens (P <0.05). Broiler chickens on CON (1276.5 g) and PEG (1243.6 g) diets had the highest HCW. Broiler chickens on GP diet had the least CCW (1075.8 g), which did not differ (P >0.05) from that of birds on ENZ (1133.2 g) and PENZ (1141.1 g) diets. However, the CCW of broilers fed PENZ was similar (P >0.05) to those fed PEG diet while the CCW of CON birds was the heaviest (1227.4 g) and did not differ (P >0.05) with the PEG diet. For water holding capacity, breast meat of birds on the PEG diet had the least value (5.223%), which did not differ (P >0.05) from that of breast meat from ENZ, GP and CON birds. The PENZ diet promoted the highest WHC (8.316%) in breast meat, which did not differ (P >0.05) from the WHC observed for ENZ, GP and CON diets. The dressing percentage, meat cooking loss, meat shear force (meat tenderness) and meat drip loss were not affected (P >0.05) by the experimental diets. There were no dietary effects on size of most internal organs except for weights of duodenum, ileum, jejunum and ceca. It was concluded that the inclusion of 10% GP treated with PEG resulted in chickens with similar HCW as those on the conventional commercial diet. The treated GP had similar WG as the CON diet, suggesting that the antinutritional effects of tannins and fibre were successfully ameliorated. However, Viscozyme® treatment did not enhance the utilization of red grape pomace in broiler chickens.

Keywords: Broilers, Condensed tannins, Fibre, Fibrolytic enzyme, Growth performance, Haemato-biochemical parameters, Meat quality, Polyethylene glycol, Red grape pomace;

4.2 Introduction

During winemaking from grapes, economic and ecological problems are caused by the large volumes of grape residues (Alonso *et al.*, 2002). Valorization of this by-product could help maintain environmental equilibrium and provide further economic benefits. One potential alternative use of grape residues from winemaking, as seen in the previous experimental chapter, is dietary incorporation into avian diets. However, the high levels of condensed tannins (20 – 30%) (Lan *et al.*, 2018) and fibre (43 - 75%) (Garcıa-Lomillo *et al.*, 2017) limit the amount of GP that could be included in poultry diets. Although GP has been proposed as a potential functional ingredient in animal feed (Brenes *et al.*, 2008) bioavailability of beneficial bioactive compounds in this by-product is rather low due to high fibre and phenolic content. According to Ebrahimzadeh *et al.* (2018) grape pomace contains high level of fibre and polymeric polyphenols such as condensed tannins that bind and precipitate both dietary and endogenous proteins, possibly reducing protein digestion and utilization in animals.

The use of natural antioxidant grape-products in diets with high polyunsaturated fatty acids content may contribute to an improved oxidative stability of meat by providing greater potential for developing quality poultry products for human consumption (Kumar *et al.*, 2015). However, the use of such natural antioxidants in animal nutrition could be limited due to the low bioavailability of grape polyphenols and might be improved by the use of exogenous enzymes. Pre-treatment of red grape pomace with fibrolytic enzymes and polyethylene glycol has the potential to increase the inclusion levels of grape pomace in chicken diets. Ebrahimzadeh *et al.* (2018) mentioned that the utilization of enzymes with capacity to hydrolyse complex cell wall might allow the use of higher doses of GP in chicken diets since high levels of fibre reduces feed intake, nutrient digestibility and utilization. Enzymatic supplementation is a technique with increasing applicability for improving the nutritional characteristic of by-products and it is widely used in animal nutrition. Cell wall

degrading enzymes can improve the *in vivo* bioavailability of phenols, which have antimicrobial and antioxidative properties. The GP cell wall is a complex network composed of 30% of neutral polysaccharides (cellulose, xyloglucan, arabinan, galactan, xylan and mannan), 20% of acidic pectin substances and 15% of insoluble proanthocyanidins, lignin, structural proteins and phenols (Pinelo *et al.*, 2006). The hydrolysis of the complex polysaccharides and polyphenols into more digestible sugars and phenols might increase the amount of beneficial bioactive substances that can be easily metabolized thus improving its nutritional value and rendering this by-product more suitable for use as a poultry feed ingredient (Chamorro *et al.*, 2014). The utilization of enzymes with capacity to hydrolyse complex cell wall and polyphenols present in GP, might allow the inclusion of higher levels of GP in chicken diets. Feed enzymes have become an important tool to increase the nutritional value of feed ingredients, reduce feed costs, and ensure environmental stewardship while maintaining or improving animal performance.

Polyethylene glycol is known for its ability to bind tannins thus freeing proteins for digestion and absorption. Indeed, Besharati and Taghizadeh (2011) reported that PEG breaks already formed tannin-protein complexes, due to its high affinity. This was also confirmed by Hlatini and Chimonyo (2018) when PEG was included in *Acacia tortilis* leaf meal diets for pigs. While the use of PEG and feed enzymes in animal diets has been widely practiced, there are no documented studies on their application to improve GP utilization and the associated effects on physiological and meat quality parameters of broiler chickens. Therefore, this study will determine the effect of PEG and Viscozyme® treatment of dietary GP on growth performance, haemato – biochemical parameters, carcass and meat quality traits of broiler chickens. It was hypothesised that the treatment of dietary GP with PEG and Viscozyme® will improve physiological parameters and meat quality traits of broiler chickens.

4.3 Materials and methods

4.3.1 Ethics statement

The procedures used to rear and slaughter broiler chickens were reviewed and approved by the Animal Research Ethics Committee, North-West University (approval no. NWU-00239-18-A5).

4.3.2 Description of the study site

The feeding trial was conducted at the North-West University Research Farm (Molelwane) as described in Chapter 3. The feeding trial was conducted in spring (September - October) and temperatures during this time ranged from 11°C to 31°C. The same broiler house described in Chapter 3 was used for this study.

4.3.3 Source of feed ingredients

Fresh red grape (*Vitis vinifera L. var. Shiraz*) pomace was supplied by Blaauwklippen wine Estate, (33.969° S; 18.844° E) (Stellenbosch, South Africa) that experiences cold and wet winters, dry and hot summers, an average daily temperature of 16.4°C, and receives average annual rainfall of 802 mm, soil types range from dark alluvial to clay. The fresh red grape pomace was air dried at room temperature and milled to pass through 1 mm sieve. Associated Chemical Enterprises (Johannesburg, South Africa), supplied PEG (Mr 4000) and the enzyme Viscozyme® L (a cellulolytic mixture of arabinase, cellulase, β-glucanase, hemicellulase and xylanase enzymes) was supplied by Sigma – Aldrich, Modderfontein, South Africa. Viscozyme® L has an enzyme activity of 100 FBG/g and a density of approximately 1.2 g/ml and completely miscible with water. A commercial feed manufacturing company, Nutroteq SA, formulated the diets while the broiler chickens were supplied by a farmer in Pretoria, South Africa.

4.3.4 Pre-treatment of red grape pomace with polyethylene glycol and Viscozyme®

Before inclusion in experimental diets, GP (5 kg per treatment) was pre-treated with aqueous solutions of PEG (5 g PEG/100 g milled GP), Viscozyme® (0.1 g enzyme/100 g milled GP), and a combination of the PEG and enzyme. For the PEG treatment, 5 kg GP was sprayed and mixed with 5000 ml of distilled water in which 250 g of PEG 4000 had been dissolved. For the enzyme treatment, 5 kg of GP was sprayed and mixed with 5000 ml of distilled water in which 4.1675 ml of Viscozyme® (density: 1.2 g/ml) had been dissolved. For the combined treatment of PEG and Viscozyme®, 250 g of PEG and 4.1675 ml Viscozyme® were both dissolved in 5000 ml distilled water, which was then sprayed on 5 kg GP. The untreated GP (5 kg) was sprayed with 5000 ml of distilled water only. The amount of distilled water used to dissolve both the PEG and enzyme was determined by trial and error with the objective of avoiding run-off liquid that would have resulted in the leaching of GP chemical components. Treated and untreated GP were stored for a period of 24 hours under room temperature to allow time for PEG and Viscozyme® to react with GP tannins and fibre, respectively. At the end of this incubation period, treated and untreated GP were then oven dried at 50˚C until constant weight, crushed to break-up lumps before being incorporated into commercial grower and finishing diets.

4.3.5 Experimental diets

Five isonitrogenous and isoenergetic experimental diets were formulated to meet the daily nutritional requirements of growing and finishing chickens according to NRC (1994) guidelines. The diets for grower and finisher phases were formulated by including treated or untreated GP at 10%, a level greater than the optimum inclusion level determined in Chapter 4. The diets were formulated as follows: 1. Commercial chicken diet without red grape pomace (CON); 2. Commercial chicken diet containing 10% red grape pomace (GP); 3.

Commercial chicken diet containing 10% red grape pomace pre-treated with PEG (5% w/w) (PEG); 4. Commercial chicken diet containing 10% red grape pomace pre-treated with Viscozyme® - L (0.1% w/w) (ENZ); and 5. Commercial chicken diet containing 10% GP pre-treated with PEG (5% w/w) and Viscozyme® - L (0.1% w/w) (PENZ). The ingredient composition of the five diets are presented in Table 4.1.

Table 4.1. Ingredient composition (g/kg as fed) of grape pomace-containing diets

	Diets[1]									
	Grower					Finisher				
Ingredients	CON	GP	PEG	ENZ	PENZ	CON	GP	PEG	ENZ	PENZ
Polyethylene glycol	0	0	5	0	5	0	0	5	0	5
Viscozyme® -L	0	0	0	0.1	0.1	0	0	0	0.1	0.1
Grape pomace	0	100	100	100	100	0	100	100	100	100
Soy oilcake	245	12	12	12	12	168	0	0	0	0
Fullfat soya	10	229	229	229	229	55	262	262	262	262
Gluten 60	5	38	38	38	38	0	0	0	0	0
Sint lysine	1.39	2.71	2.71	2.71	2.71	1.93	1.52	1.52	1.52	1.52
Methionine	1.42	0.8	0.8	0.8	0.8	1.51	0.97	0.97	0.97	0.97
Threonine	0	0.01	0.01	0.01	0.01	0.1	0	0	0	0
Yellow maize	709	589	589	589	589	751	601	601	601	601
Feed lime	14.6	13	13	13	13	12.5	11.3	11.3	11.3	11.3
Monocalcium phosphate	7	7.9	7.9	7.9	7.9	2.2	2.2	2.2	2.2	2.2
Salt-fine	3.29	3.35	3.35	3.35	3.35	2.78	3.11	3.11	3.11	3.11
Sodium bicarbonate	1.59	1.45	1.45	1.45	1.45	1.91	1.28	1.28	1.28	1.28
Axtra phytase	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline	0.8	0.8	0.8	0.8	0.8	0	0	0	0	0
Salinomycin	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0
Olaquindox	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2
Premix	0.5	0.5	0.5	0.5	0.5	2.5	2.5	2.5	2.5	2.5
Zinc bacitracin	0	0	0	0	0	0.5	0.5	0.5	0.5	0.5
Oil crude soya	0	0	0	0	0	0	13.17	13.17	13.17	13.17

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme®- L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme®- L (0.1% w/w).

4.3.6 Chemical analysis

The formulated diets (CON, GP, PEG, ENZ and PENZ) were sampled and milled for the determination of dry matter, metabolizable energy, crude protein, crude fat, crude fibre, organic matter and minerals as previously described in Chapter 3.

4.3.7 Experimental design

A total of four hundred, day old Cobb 500 broiler chickens were randomly and evenly allocated to a total of 40 pens (experimental units) in a broiler unit in a completely randomized design. The dietary treatments were replicated 8 times with each pen carrying 10 chickens. The pens (measuring 3.5 long × 1.0 wide × 1.85 high m) were designed to meet the animal welfare standards for chickens. The day-old broiler chicks were fed a commercial starter diet from Nutri-feeds, Potchefstroom, South Africa, until 10 days before being adapted to the experimental diets for 3 days such that measurements commenced on day 14 of age.

4.3.8 Feeding and broiler management

Dietary treatments and fresh water were provided *ad libitum* and average daily feed intake was measured from week 3 to week 6. Feed intake was measured daily and body weight was measured weekly. All birds from the forty pens were weighed at the beginning of the trial (initial body weight) and subsequently weighed weekly (CBK Bench Scales, Scaletec, Cape Town, South Africa). The feed offered was weighed before feeding and refusals were collected each morning before feeding. Average weekly feed intake (AWFI) and average weekly gain (AWG) were used to calculate feed conversion ratio (FCR).

4.3.9 Blood collection and analysis

Blood collection and analysis were performed as described in Chapter 3. Haematological parameters (erythrocyte count, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell (RDW), haemoglobin, neutrophils (NEU), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOSO), reticulolytes (RETIC), white blood cell (WBC) and basophils (BASO) were analysed using the IDEXX LaserCyte Dx Haematology Analyser equipment (IDEXX Laboratories, Kyalami gardens, Midrand South Africa) while serum biochemical parameters (glucose, calcium, creatinine, total protein(TPr), albumin, phosphorus, globulin, total bilirubin (TBIL), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), gamma-glutamyl transferase (GGT), cholesterol, amylase and lipase) were analysed using IDEXX Catalyst One Chemistry Analyzer (IDEXX Laboratories, Kyalami gardens, Midrand South Africa)at North-West University Animal Science laboratory (Mafikeng, SA).

4.3.10 Slaughter procedures

At 6 weeks of age, all broiler chickens were starved for 24 hours and taken to Rooigrond poultry abattoir (North West province, South Africa) for slaughter. At the abattoir, all the chickens were stunned and live-hanged onto a movable metal rack that holds them upside down by their feet. Chickens were then slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding stopped. The chickens were then defeathered and the carcases were taken to the NWU Meat Science Laboratory for measurements.

4.3.11 Carcass traits and internal organs

After slaughter, all carcasses were packed in labelled plastic bags. Thereafter, carcasses were eviscerated and carcass traits measured. The following were removed and weighed: gizzards,

livers, proventriculi, breasts, thighs, drumsticks and wings. Length and weights of duodenum, ileum, jejunum, ceca and large intestines were also measured and recorded. Hot carcass weights (HCW) were measured immediately after slaughter while cold carcass weight (CCW) was measured after the carcasses had been chilled at 6°C for 24 h. Dressing out percentage was calculated as the proportion of HCW on slaughter weights.Breast (pectoralis major muscle) samples were carefully removed 24 hours after slaughter for evaluation of meat quality traits. Breast samples were then vacuum packed and frozen (−20°C) pending meat quality analysis.

4.3.12 Meat quality measurements

Meat pH and temperature, meat colour, water holding capacity, drip loss, cooking loss and meat tenderness were measured as described in Chapter 3.

4.3.12.1 Meat shelf life

A randomly selected breast meat sample from each replicate pen was used for the determination of broiler meat shelf life at room temperature. The breast meat samples were placed in labelled foil trays and stored on top of the table at North-West University Meat Science laboratory. Meat pH and color (lightness, redness and yellowness, chroma and hue angle) were then recorded daily for a period of 4 days.

4.3.13 Statistical analysis

Data for each parameter collected per replicate pen were averaged first before statistical analysis. The NORMAL option in the Proc Univariate statement was used to test for normality of measured parameters before analysis of variance. Weekly feed intake, weight

gain and feed conversion ratio data were analysed using the repeated measures analysis SAS (2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

where, Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed.

Overall feed intake, weight gain, feed conversion ratio, blood parameters, carcass characteristics and meat quality data were analysed using the general linear model procedure of SAS (2010). The linear statistical model employed was as follows:

$$Y_{ik} = \mu + D_i + E_{ik}$$

Where, Y_{ik} = dependant variable, μ = population mean, D_i = effect of diets and E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

4.4 Results

The experimental diets were formulated to be isoenergetic and isonitrogenous. The dry mater content of CON diet was generally lower than the other experimental diets.

Table 4.2. Chemical composition (g/kg DM, unless otherwise stated) of red grape pomace-containing diets

	Diets[1]									
	Grower					Finisher				
[2]Parameters	CON	GP	PEG	ENZ	PENZ	CON	GP	PEG	ENZ	PENZ
Dry matter (g/kg)	893.7	906.7	906.7	906.7	906.7	888.6	904.2	904.2	904.2	904.2
ME (MJ/kg)	119.1	119.0	119.0	119.0	119.0	122.0	122.0	122.0	122.0	122.0
Protein	177.1	170.1	170.1	170.1	170.1	160.0	159.97	159.97	159.97	159.97
Fat	33.50	72.60	72.60	72.6	72.6	42.7	89.8	89.8	89.8	89.8
Fibre	25.0	74.6	74.6	74.6	74.6	35.2	80.7	80.7	80.7	80.7
OM	844.1	857.3	857.3	857.3	857.3	849.0	862.5	862.5	862.5	862.5
Calcium	8.2	8.2	8.2	8.2	8.2	6.5	6.5	6.5	6.5	6.5
Phosphorus	5.0	4.7	4.7	4.7	4.7	3.4	3.28	3.28	3.28	3.28
Chloride	2.8	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w).

[2]Parameters: ME = metabolisable energy; OM = organic matter.

Repeated measures analysis showed no significant (P >0.05) week × diet interaction effects on AWFI, AWG and FCR. Table 4.3 below shows that there were no significant (P >0.05) dietary influences on overall feed intake (g/bird) and overall FCR. The CON (1351.4 g/bird) diet promoted the highest overall WG, which did not differ (P >0.05) from PEG, ENZ and PENZ diets, however, GP birds had the least weight gain (1188.9 g/bird).

Table 4.3. Effect of treating red grape pomace with polyethylene glycol and fibrolytic enzyme mixture on overall feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) of broiler chickens

	[1]Diets					[2]SEM	[3]Significance
	CON	GP	PEG	ENZ	PENZ		
Overall FI (g/bird)	2957.5	2844.9	2931.6	2930.8	2913.6	40.52	NS
Overall WG (g/bird)	1351.4^{b}	1188.9^{a}	1308.9^{ab}	1234.9^{ab}	1271.3^{ab}	35.69	*
Overall FCR	2.195	2.398	2.244	2.238	2.291	0.057	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w).

[2]SEM: Standard error of the mean

[3]Significance: NS = not significant; * = P <0.05.

With the exception of Mean corpuscular volume (MCV), all haematological parameters were not ($P > 0.05$) influenced by dietary treatments (Table 4.4). Broilers fed ENZ had higher MCV (34.87 fL) when compared to those on CON (27. 66) which did not differ from GP and PEG diets. Broilers on PENZ had similar effects ($P > 0.05$) as those on ENZ and PEG diets.

Table 4.4. Effect of treating red grape pomace with polyethylene glycol and fibrolytic enzyme on haematological parameters of broiler chickens

	[1]Diets						
[2]Parameters	CON	GP	PEG	ENZ	PENZ	[3]SEM	[4]Significance
Erythrocyte C ($\times 10^{12}$/L)	1.261	1.471	1.328	1.048	1.229	0.309	NS
Haematocrit (L/L)	5.481	5.725	5.406	6.188	5.469	0.565	NS
Haemoglobin (g/dl)	9.413	9.888	10.075	9.744	9.519	0.267	NS
MCV (fl)	27.66^{a}	27.70^{a}	27.89^{ab}	34.87^{c}	33.48^{bc}	1.432	**
MCH (pg)	49.17	51.98	53.31	52.52	59.09	6.195	NS
RDW ($\times 10^{9}$/L)	40.81	40.69	39.58	38.74	36.73	1.106	NS
RETIC (K/μL)	235.2	186.06	178.3	85.96	86.2	50.87	NS
WBC ($\times 10^{9}$/L)	30.07	82.46	115.3	59.74	57.38	25.53	NS
LYMP ($\times 10^{9}$/L)	19.85	27.56	93.92	52.11	50.71	26.14	NS
NEU ($\times 10^{9}$/L)	3.538	3.974	3.728	4.153	4.558	0.494	NS
MONO ($\times 10^{9}$/L)	1.906	2.014	2.360	2.477	1.848	0.356	NS
EOS ($\times 10^{9}$/L)	0.553	0.659	0.823	0.837	0.790	0.134	NS
BASO ($\times 10^{9}$/L)	0.112	0.116	0.144	0.16	0.156	0.020	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w); [2]Parameters: Erythrocyte C = Erythrocyte count; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; RDW=red blood cell; NEU = Neutrophils; LYMP = lymphocytes; MONO = monocytes; EOSO = eosinophils; RETIC = reticulolyte; WBC = white blood cell; BASO = basophils; [3]SEM: Standard error of the mean; [4]Significance: NS = not significant; ** = $P < 0.05$.

With the exception of phosphorus, all serum biochemical parameters were not significantly affected by dietary treatments. Broilers on GP had the least phosphorus which did not differ (P >0.05) with CON and PENZ diets. Broilers on ENZ had the highest (4.74 mmol/L) which did not differ from PEG and CON diets.

Table 4.5. Effect of treating red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on serum biochemical parameters of broiler chickens

	[1]Diets						
[2]Parameters	CON	GP	PEG	ENZ	PENZ	[3]SEM	[4]Significance
Glucose (mmol/L)	8.061	6.696	8.544	8.539	8.060	1.241	NS
Creatinine (µmol/L)	13.81	10.94	18.00	15.75	15.38	2.416	NS
Urea (mmol/L)	0.656	0.663	0.706	0.688	0.700	0.021	NS
Phosphorus (mmol/L)	3.88^{ac}	3.30^{a}	4.54^{bc}	4.74^{bc}	3.64^{ab}	0.300	*
Calcium (mmol/L)	2.241	2.181	2.203	2.548	2.563	0.241	NS
Total protein (g/L)	51.38	51.88	55.11	59.38	62.06	3.776	NS
Albumin (g/L)	19.25	18.88	21.38	23.88	22.19	1.990	NS
Globulin (g/L)	33.44	33.13	31.50	35.44	39.31	2.465	NS
ALT (U/L)	53.81	55.13	50.00	71.50	64.44	6.913	NS
ALKP (U/L)	696.1	508.1	566.7	640.6	676.9	94.42	NS
GGT (U/L)	17.06	15.81	18.88	15.75	12.94	1.762	NS
Total bilirubin (µmol/L)	11.81	15.38	14.19	20.94	18.50	2.870	NS
Amylase (U/L)	547.9	461.4	472.3	564.3	516.6	42.81	NS
Lipase (U/L)	317.3	298.6	371.3	465.3	445.63	60.82	NS
Cholesterol (mmol/L)	6.236	6.143	6.037	6.573	6.681	0.450	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w); [2]Parameters: ALT = Alanine transaminase; ALKP = Alkaline phosphate; GGT = Gamma glutamyl transferase [3]SEM: Standard error of the mean; [4]Significance: NS = not significant; * = $P < 0.05$.

Table 4.6 shows that there were significant dietary effects on slaughter weight, HCW, CCW and WHC of broiler chickens (P <0.05). The slaughter weights of CON, PEG, ENZ and PENZ chickens did not differ (P >0.05). However, GP diet promoted the least slaughter weight (1468.4 g) in chickens. Broiler chickens on CON (1276.5 g) and PEG (1243.6 g) diets had bigger HCW, which did not differ. However, GP promoted the least (1120.6 g) HCW, which was similar (P >0.05) to that of birds fed ENZ and PENZ diets. Meanwhile, the HCW of PEG, ENZ and PENZ chickens did not differ (P >0.05). Broilers on the CON (1227.4 g) and PEG (1210.0 g) diets had higher CCW compared to GP, ENZ and PENZ fed chickens, whose CCW did not differ. Diets significantly affected the WHC of breast meat with PENZ promoting the highest WHC (8.316 %) and PEG promoting the least (5.223 %).

Table 4.6. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on meat quality traits of broiler chickens

	[1]Diets						
[2]Parameters	CON	GP	PEG	ENZ	PENZ	[3]SEM	[4]Significance
HCW (g)	1276.5^{c}	1120.6^{a}	1243.6^{bc}	1177.9^{ab}	1181.4^{ab}	18.38	***
CCW (g)	1227.4^{c}	1075.8^{a}	1210.0^{bc}	1133.2^{a}	1141.1^{ab}	18.53	***
Slaughter weight (g)	1653.2^{b}	1468.4^{a}	1604.4^{ab}	1523.0^{ab}	1564.9^{ab}	35.53	**
Dressing %	77.23	76.50	77.72	77.71	75.63	1.499	NS
Cooking Loss (%)	19.19	22.72	21.32	22.91	23.31	1.060	NS
Shear Force (N)	4.843	4.403	4.863	5.645	4.891	0.366	NS
WHC (%)	7.819^{ab}	7.577^{ab}	5.223^{a}	5.604^{ab}	8.316^{b}	0.763	*
Drip loss (%)	16.22	18.34	14.89	16.55	16.14	0.916	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w); [2]Parameters: HCW = hot carcass weight; CCW = cold carcass weight; W4 = slaughter weight; CL = cooking loss; WHC = water holding capacity; [3]SEM: Standard error of the mean; [4]Significance: NS = not significant; *** = $P < 0.001$

[a,b] In a row, dietary treatment means with common superscripts do not differ ($P > 0.05$).

Table 4.7 shows that there were no significant dietary effects on weights of wings (5.451 – 5.664 % HCW), thighs (7.605 – 8.640 % HCW) and drumsticks (6.256 – 7.233 % HCW) of broiler chickens. However, diets affected size of breasts (P <0.001). Broilers on CON diet had the heaviest breasts (22.484 % HCW), which did not differ (P >0.05) with those for chickens on PEG diet (21.308 % HCW). However, the breast weights of broilers fed the PEG diet was similar (P >0.05) to those fed GP, ENZ and PENZ diets. The size of the back, thighs and drumsticks were not significantly different (P >0.05). The length of wings significantly differed (P <0.05) across diets. Broiler chickens fed the CON diet had the longest wing length (18.753 cm), which did not differ from those on the PEG (18.266 cm), ENZ (18.230 cm) and PENZ (18.491 cm) diets. Chickens on the GP diet had the shortest wing length (17.406 cm), which did not differ (P >0.05) from that of chickens on PEG, ENZ and PENZ diets.

Table 4.7. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on carcass characteristics of broiler chickens

	[1]Diets					[2]SEM	[3]Significance
	CON	ENZ	GP	PEG	PENZ		
Weights (% HCW[4])							
Breasts	22.484[b]	20.812[a]	20.187[a]	21.308[ab]	20.341[a]	0.363	***
Wings	5.451	5.664	5.541	5.936	5.612	0.127	NS
Thighs	7.605	8.541	8.640	8.336	8.055	0.521	NS
Drumsticks	6.526	6.556	6.498	6.256	7.233	0.363	NS
Lengths (cm)							
Backs	18.257	17.416	19.425	17.951	18.168	0.498	NS
Wings	18.753[b]	18.230[ab]	17.406[a]	18.266[ab]	18.491[ab]	0.320	*
Thighs	9.693	9.372	9.710	9.505	9.293	0.131	NS
Drumsticks	10.672	10.311	11.498	10.417	10.613	0.351	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w); [2]SEM: Standard error of the mean; [3]Significance: NS = not significant; *** = $P < 0.001$, * = $P < 0.05$; [4]HCW = Hot carcass weight.

[a,b] In a row, dietary treatment means with common superscripts do not differ ($P > 0.05$).

For internal organs, Table 4.8 shows that there were no dietary effects (P >0.05) on size of livers (2.217 – 2.306% HCW), gizzards (2.302 – 2.470% HCW), hearts (0.641 – 0.738% HCW), proventriculus (0.512 – 0.552% HCW), spleens (0.113 – 0.155% HCW), pancreas (0.241 – 0.282% HCW), LI (0.216 – 0.706% HCW) and lungs (0.645 – 0.726% HCW) of broiler chickens. However, dietary effects (P <0.05) were observed for weights of duodenum, ileum, jejunum and ceca. The CON diet promoted the smallest duodenum weights (0.663% HCW) while chickens on GP, PEG ENZ and PENZ diets had bigger weights that did not differ (P >0.05). Chickens on the CON diet had the lowest ileum weight (1.356% HCW), which did not differ from those fed PEG, ENZ and PENZ diets. Birds on the GP diets had the highest ileum weight (1.657% HCW), which did not differ from those fed PEG, ENZ and PENZ diets. CON experimental diet promoted lower (P <0.05) jejunum weights (1.376% HCW) compared to GP, PEG, ENZ and PENZ diets, which did not differ. The broilers on CON (0.822% HCW) and PEG (1.041% HCW) diets had the lowest ceca weights while birds on GP diets had the highest ceca weight (1.397% HCW), which did not differ from PENZ diet.

Table 4.8. Effect of grape pomace-containing diets treated with polyethylene glycol and a fibrolytic enzyme mixture on size of internal organs (% HCW[5]) of broiler chickens

	[1]Diets						
[2]Parameters	CON	ENZ	GP	PEG	PENZ	[3]SEM	[4]Significance
Livers	2.306	2.223	2.217	2.267	2.227	0.043	NS
Gizzards	2.302	2.470	2.343	2.326	2.445	0.072	NS
Hearts	0.641	0.695	0.686	0.738	0.687	0.022	NS
Proventriculus	0.512	0.541	0.552	0.547	0.536	0.017	NS
Spleens	0.113	0.155	0.118	0.126	0.122	0.013	NS
Pancreas	0.255	0.246	0.256	0.282	0.241	0.014	NS
Duodenum	0.663^{a}	0.842^{b}	0.827^{b}	0.820^{b}	0.797^{b}	0.027	***
Ileum	1.356^{a}	1.542^{ab}	1.657^{b}	1.477^{ab}	1.525^{ab}	0.050	**
Jejunum	1.376^{a}	1.613^{b}	1.716^{b}	1.590^{b}	1.675^{b}	0.045	***
LI	0.706	0.370	0.382	0.216	0.393	0.112	NS
Ceca	0.822^{a}	1.137^{b}	1.397^{c}	1.041^{ab}	1.232^{bc}	0.056	***
Lungs	0.651	0.645	0.680	0.726	0.667	0.031	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w); [2]Parameters: LI = large intestines; [3]SEM: Standard error of the mean; [4]Significance: NS = not significant; *** = $P < 0.001$; [5]HCW = Hot carcass weight

[a,b] In a row, dietary treatment means with common superscripts do not differ ($P > 0.05$).

For meat quality traits parameters, Table 4.9 shows that the diets had no significant effect on meat temperature 24 hours after slaughter (14.575 – 16.55 °C), meat pH (6.760 – 6.95), L* (48.51 – 49.849), a* (1.474 – 1.5133), b* (12.230 – 14.504), chroma (12.29 – 14.54) and hue angle (0.843 – 1.276) of broiler chickens.

Table 4.9. Breast meat quality traits of broiler chickens fed diets containing red grape pomace treated with polyethylene glycol and a fibrolytic enzyme mixture.

	[1]Diets						
[2]Parameters	CON	GP	PEG	ENZ	PENZ	[3]SEM	[4]Significance
Temperature (°C)	14.575	16.55	14.825	15.675	15.775	0.48201	NS
Meat pH	6.95425	6.76025	6.9215	6.90875	6.8205	0.085506	NS
L*	48.58775	49.8495	49.7605	48.51475	49.4975	0.614469	NS
a *	1.474327	1.478478	1.513333	1.485541	1.498862	0.01368	NS
b*	13.7455	12.23025	14.504	13.4445	13.81075	0.57055	NS
Chroma	13.84742	12.29914	14.54488	13.53897	13.8688	0.577798	NS
Hue Angle	1.27675	1.04825	0.8435	1.12425	1.00275	0.179942	NS

[1]Diets: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme- L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and viscozyme- L (0.1% w/w); [2]Parameters: L* = lightness of the meat; a* = meat redness; b* = yellowness of the meat; [3]SEM: Standard error of the mean; [4]Significance: NS = not significant

With the exception of meat pH and temperature, all meat quality parameters measured at room temperature over 4 days were not significantly (P >0.05) affected by dietary treatments (Figures 4.1 – 4.7). The breast meat pH for broiler chickens on CON diets decreased, while that of breast meat from birds on GP, PEG, ENZ and PENZ diets increased over 4 days. The lightness (L*) and yellowness (b*) of the breast meat decreased while breast meat redness (a*) increased with time for all the diets. The chroma and hue angle of breast meat declined over 4 days for all diets.

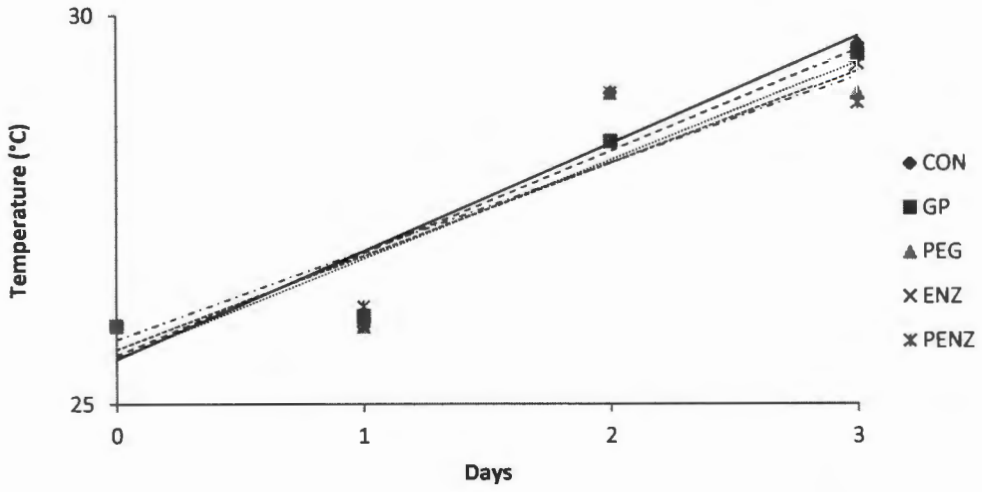

Figure 4.1. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on the stability of breast meat temperature (°C) upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)]

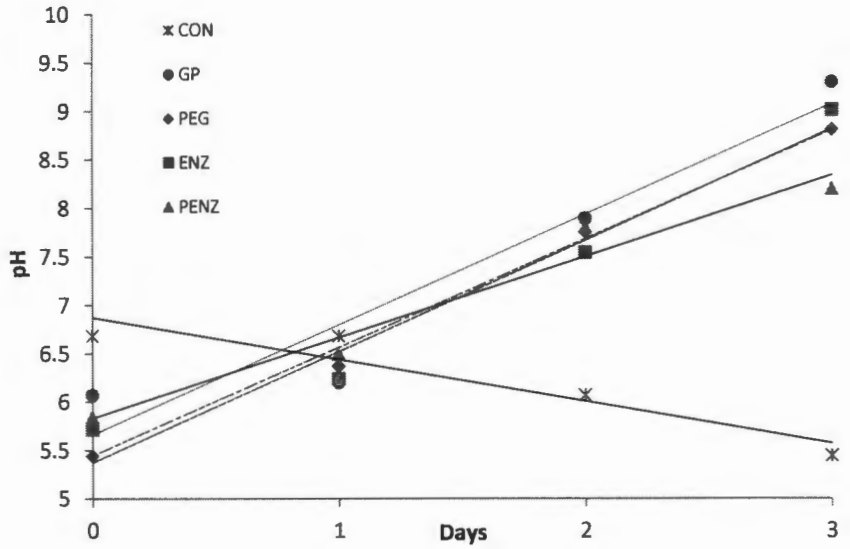

Figure 4.2. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on the stability of breast meat pH upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

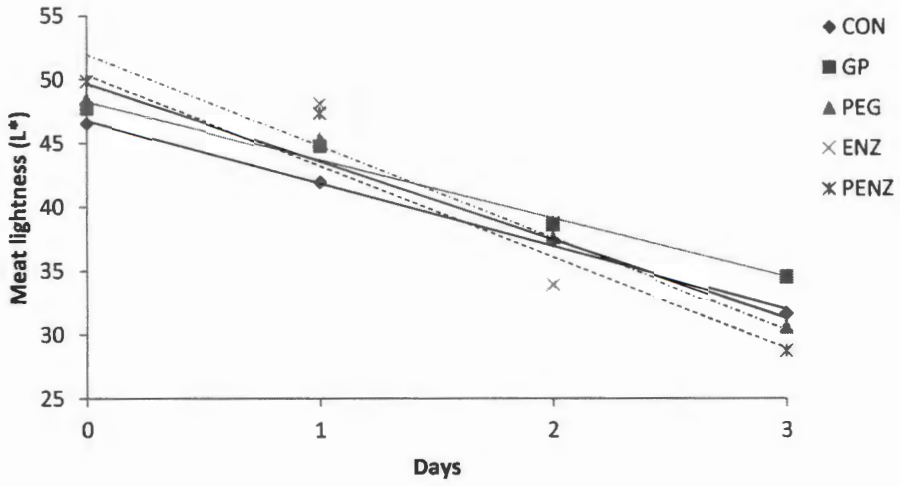

Figure 4.3. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on stability of breast meat lightness upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

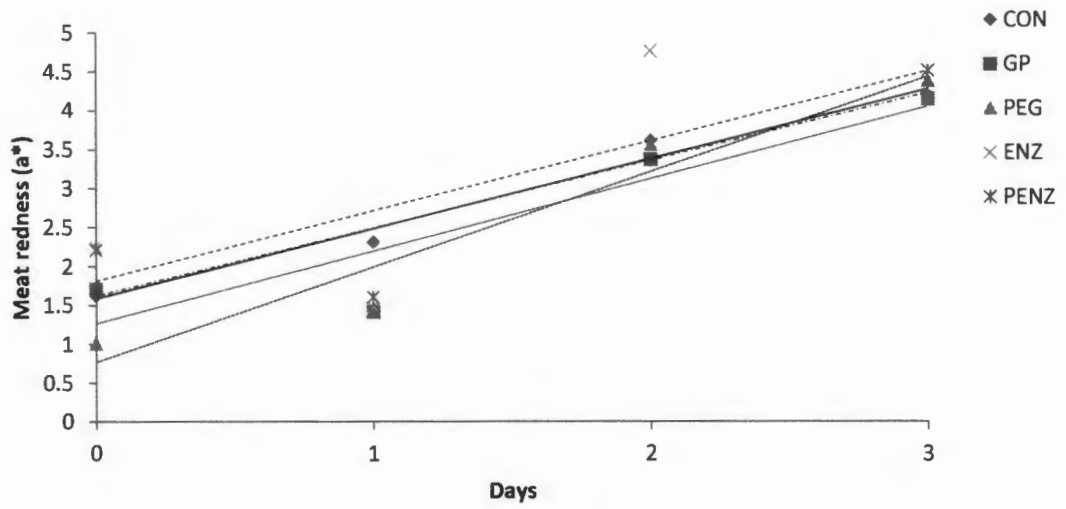

Figure 4.4. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on stability of breast meat redness upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

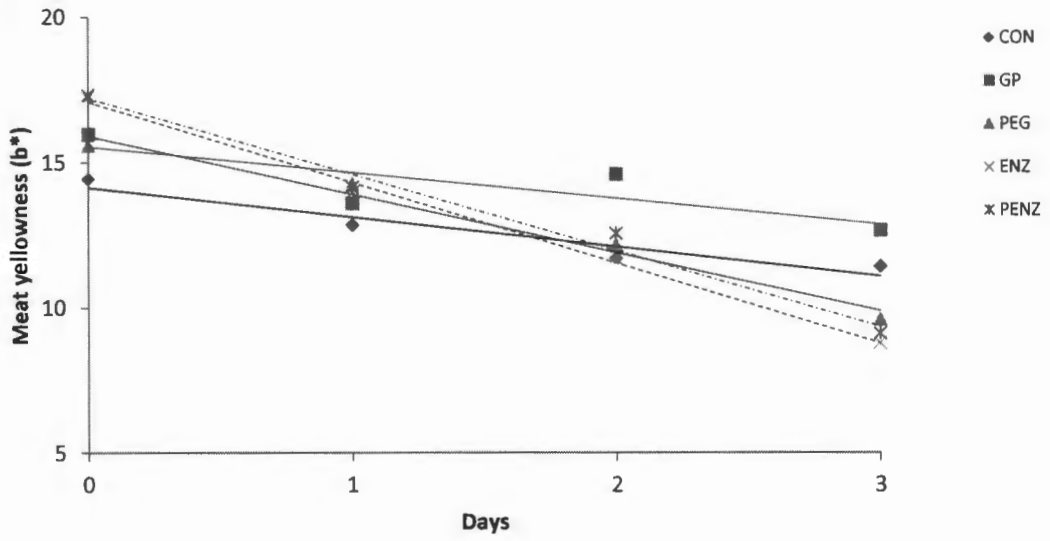

Figure 4.5. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on stability of breast meat yellowness upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

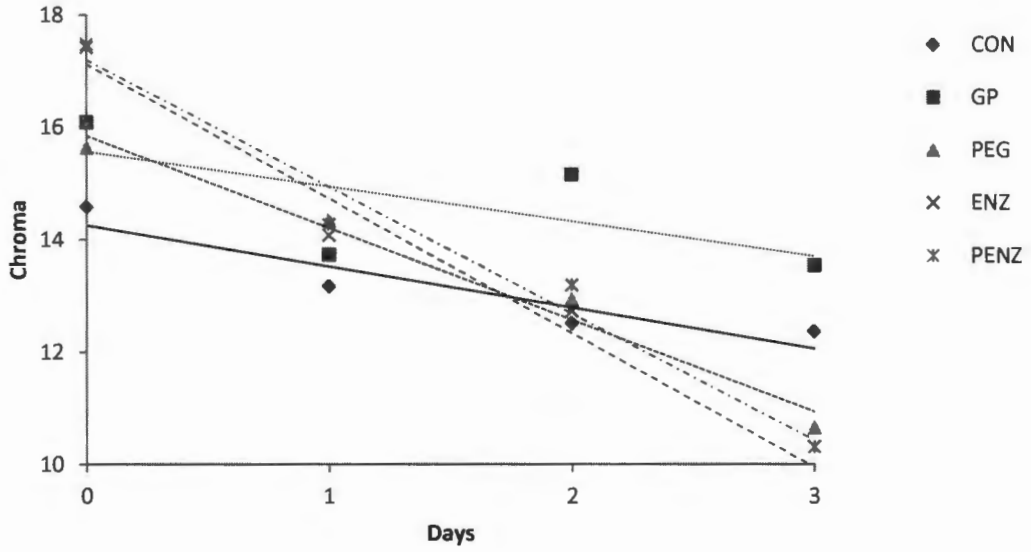

Figure 4.6. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on the stability of breast meat chroma upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

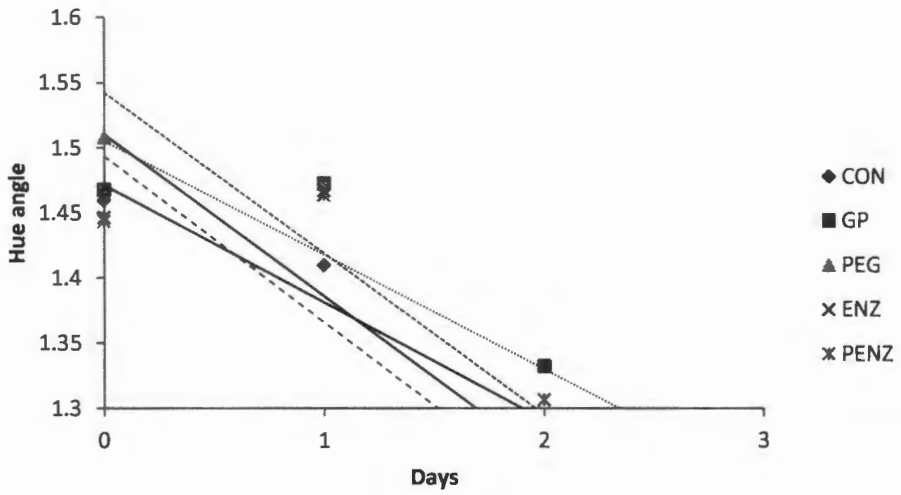

Figure 4.7. Effect of treating dietary red grape pomace with polyethylene glycol and a fibrolytic enzyme mixture on stability of breast meat hue angle upon storage at room temperature for 4 days. [***Diets***: CON = commercial chicken diet without grape pomace; GP = commercial chicken diet containing 10% grape pomace; PEG = commercial chicken diet containing 10% grape pomace pre-treated with polyethylene glycol (5% w/w); ENZ = commercial chicken diet containing 10% grape pomace pre-treated with Viscozyme® - L (0.1% w/w); PENZ = commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w)].

4.5 Discussion

The use of polyethylene glycol (PEG) and feed enzymes on animal feed diets has been widely practiced; however, there are no documented studies on their application to improve utilization of dietary GP by broiler chickens. The combined effects of PEG and feed enzymes on physiological and meat quality parameters of broiler chickens have not been reported in literature. The combination of PEG and Viscozyme® has the potential to increase the inclusion level of GP in broiler diets thus reducing feed costs, improve broiler meat quality and ensure good environmental stewardship.

Results from this study suggest that the inclusion of GP in chicken diets at 10% had no effect on overall feed intake when treated with polyethylene glycol and or enzyme. These results are in agreement with Chamorro *et al.* (2014) who found no effect on feeding diets containing GP treated with carbohydrase enzymes complex and tannase on feed intake of male broiler Cobb chicks. In addition, Ebrahimzadeh *et al.* (2018) reported that addition of up to 10% GP in broiler chicken diets had no effect on average daily feed intake. Furthermore, Kara *et al.* (2016) concluded that grape pomace addition of up to 6 % in laying hen diets did not affect their feed intake. In the contrast, Sayago-Ayerdi *et al.* (2009) stated that inclusion of GP over 6 % reduce feed intake. In this study it was expected that the broilers fed commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w) (PENZ) will have the highest overall feed intake. Viscozyme® has the capacity to hydrolyze complex plant cell walls, while polyethylene glycol binds to tannins thereby neutralizing their anti-nutritional effects. It was, therefore, anticipated that GP intake would be enhanced by the treatments. However, there were no differences on feed intake in all the experimental diets.

The use of PEG and Viscozyme® had an effect only on overall weight gain of broiler chickens. The treated GP had similar overall WG as the CON diet suggesting that the antinutritional effects of tannins and fibre were successfully ameliorated. These findings are in contrast with the results reported by Chamorro *et al.* (2015) when he studied the influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed 10 % grape pomace. This is, however, expected as Chamorro *et al.* (2015) only used feed enzymes, which were not combined with PEG suggesting that the negative nutritional effects of tannins were not ameliorated. Recent results from Ebrahimzadeh *et al.* (2017) showed that dietary inclusion of 10% GP with or without tannase enzyme treatment did not affect the chick growth performance. Grape pomace contains high level of fibre and polymeric polyphenols such as procyanidins with capacity to bind and precipitate both dietary and endogenous proteins. As such, the incorporation of high levels of GP in chicken diets might impair nutrient digestion and growth. Even though feed intake was not affected by diets, it is evident that the feed was successfully converted into useful nutrients. The effect of GP diets on growth performance shows that PEG and Viscozyme® treatment increased the amount of nutrients released in the intestines. Generally, high tannin content diets reduce growth performance, therefore, in this study it was observed that the untreated GP reduced overall WG when compared to the commercial broiler diet (CON). However, the treated GP had similar overall WG as the commercial broiler diet (CON) suggesting that the antinutritional effect of tannins and fibre in GP were successfully alleviated.

Experimental diets had no significant effect on haematological and serum biochemical parameters of broiler chickens, which fell within the normal range for chickens. This suggests that polyethylene glycol and Viscozyme® treatment of broiler diets did not influence the physical and pathophysiological status of broilers. However, MCV and phosphorus levels were higher on Viscozyme® treated diets. A study by Aditya *et al.* (2018) on

supplementation of GP (*Vitis vinifera*) in broiler diets reported lower serum total cholesterol levels in GP supplemented groups. Kara (2015) reported no change of serum triglyceride and total cholesterol levels when supplementing laying hens with grape pomace.

The grape pomace-containing diets treated with PEG and Viscozyme® had no effect on weight of internal organs except for intestinal parts (duodenum, ileum, jejunum and ceca). Theoretically, high fibre diets are expected to increase the length of the intestines as an adaptation mechanism to handle additional fibre. Polyethylene glycol and cell wall degrading enzymes were introduced to aid the digestion process, however, from these results it is clear that digestion was still low in broiler chickens. The relative weights of livers, gizzards and pancreas were not affected by the diets. Although these results were not expected, they are in agreement with Brenes *et al.* (2008) who reported that broilers fed diets containing GP concentrate showed no significant differences in the relative weight of internal organs compared with the control groups. The expectation was that untreated and enzyme-treated GP would still contain phenolics that require detoxification by the liver upon absorption from the digestive tract leading to atrophy of the organ. For untreated and PEG-treated GP the fibre levels would still be high leading to increase in the size of the gizzard.

There were significant effects of the diets on the carcasses and slaughter weights of broiler chickens. The diet with untreated GP had the least slaughter weight compared to the treated GP diets. These findings, therefore, suggest that treating GP with PEG and Viscozyme® results in bigger carcasses and are explained by the amelioration of the antinutritional effects of tannins and fibre by PEG and enzyme, respectively. These results are in contrast with those of Brenes *et al.* (2008) who reported that the inclusion of graded concentrations of GP and vitamin E did not affect carcass weight in chickens

Furthermore, the meat temperature, meat pH and meat color were not altered by the experimental diets, indicating the ineffectiveness of PEG and Viscozyme® in this regard. This finding corroborates Carpenter *et al.* (2007)'s findings that the addition of grape seed extract on raw pork patties does not affect lightness, redness and yellowness. Aditya *et al.* (2018) reported that at 5 days after storage no effect on lightness values was observed, however, after 10 days of storage lightness values showed a quadratic effect on broiler meat. In contrast, Kasapidou *et al.* (2016) reported that GP affected redness and resulted in paler broiler meat. The meat color (redness) was, however, expected to be influenced by the diets given that GP contains anthocyanins that cause meat pigmentation.

For meat stability (shelf life), all meat quality parameters measured at room temperature over 4 days were not significantly affected by dietary treatments with the exception of meat pH and temperature. For the CON diets the meat pH decreased over time, however, on the GP diets meat pH increased with time. Generally the increase in meat pH is associated with improved meat quality, however the increase of pH over time also suggest microbial proliferation. These results are in agreement with Gai *et al.* (2015) who evaluated the effect of red grape pomace extract on the shelf life of refrigerated rainbow trout minced muscle over 6 days.

4.6 Conclusions

It was concluded that the inclusion of 10% GP treated with PEG resulted in chickens with similar HCW as those on the conventional commercial diet. The untreated GP reduced weight gain and FCR. However, the treated GP had similar weight gain and FCR as the CON diet suggesting that the antinutritional effects of tannins and fibre were successfully ameliorated.

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5 CHAPTER FIVE – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

Growth of the human population has resulted in an increase in competition for food between humans and livestock thus necessitating the search for alternative feed resources for animals. The poultry industry provides affordable and healthy animal protein to feed the growing human population. This is because broilers have a short generation interval, high reproduction rate and high nutrient conversion efficiency. The broiler strain of interest in this study, Cobb 500, has shown several attributes such as disease resistance, fast growth rates and tolerance to harsh environmental conditions (Brenes *et al.*, 2008).

According to Lu and Foo (1999), the vinification process produces large quantities of grape pomace (GP) whose disposal poses an environmental challenge yet this waste product contains some beneficial bioactive compounds that can be exploited in a variety of ways. Revalorization of GP as a nutraceutical in chicken diets would make this waste product an environmentally sustainable, low-cost alternative feed ingredient. This study was, therefore, designed to evaluate the utility of this waste product as a component of broiler diets through a series of experiments. Initially, a feeding trial was conducted to determine the effect of graded levels of dietary red grape pomace on growth performance, haematology, serum biochemistry, carcass characteristics and meat quality parameters of Cobb 500 broiler chickens. The GP was incorporated in commercial broiler diet at GP0 = commercial chicken diet without GP; GP25 = commercial chicken diet containing 2.5% GP; GP45 = commercial chicken diet containing 4.5% GP; GP55 = commercial chicken diet containing 5.5% GP; and GP75 = commercial chicken diet containing 7.5% GP, producing five isonitrogenous and isoenergetic diets which were fed to four hundred Cobb 500 broiler chickens. Feed intake,

weight gain, haematology, serum biochemistry, carcass characteristics and meat quality parameters were determined. Dietary inclusion of red grape pomace up to 7.5% promoted similar levels of weight gain as the commercial control diet while enhancing meat redness despite reduction in feed intake. Higher inclusion levels of GP (5.5% and 7.5%) promoted higher feed conversion ratio compared to the commercial control diet. The hypothesis that including GP in Cobb 500 broiler diets will improve physiological, carcass, and meat quality traits was accepted for FCR and meat redness. The use of natural antioxidant grape-products in diets with high polyunsaturated fatty acids content may contribute to an improved oxidative stability of meat by providing greater potential for developing quality poultry products for human consumption (Kumar *et al.*, 2015). In addition, GP polyphenols have been reported to boost the levels of glutathione and catalase molecules that enhance immune function (Ruberto *et al.*, 2007). Inclusion of GP in commercial chicken diets beyond this maximum (7.5%) may be necessary to further lower feed costs and promote greater intake of beneficial bioactive compounds.

The observed reduction in feed intake in the first study indicates that the amount of GP that can be incorporated in broiler diets may be limited by antinutritional factors such as fibre and condensed tannins. Indeed, Brenes *et al.* (2008) mentioned that the fibre in GP may negatively affect nutrient digestion and absorption while condensed tannins can bind to proteins thus, reduce their availability. A number of methods have been used to reduce the detrimental and toxic effects of tannins in order to improve the nutritive value of tannin-rich feedstuffs (Mlambo *et al.*, 2011). These methods include the use of tannin-binding agents such as sodium hydroxide, wood ash, and polyethylene glycol. Fibrolytic enzymes are also a strategy of choice to improve fibre utilization in non-ruminants diets. In order to improve the utilization of GP beyond optimum level (7.5 %) in Cobb 500 broiler chickens, polyethylene glycol, a tannin-binding agent, and Viscozyme® - L, a mixture of fibrolytic enzymes, were

used to pre-treat GP before incorporation into diets. The second study of this thesis, therefore, evaluated the effect of pre-treating GP with polyethylene glycol (PEG) and Viscozyme® (a cellulolytic mixture of arabinase, cellulase, β-glucanase, hemicellulase and xylanase enzymes) on growth performance, carcass characteristics and meat quality parameters of Cobb 500 broiler chickens. Five dietary treatments were formulated as follows: 1. Commercial chicken diet without red grape pomace (CON); 2. Commercial chicken diet containing 10% red grape pomace (GP); 3. Commercial chicken diet containing 10% red grape pomace pre-treated with polyethylene glycol (5% w/w) (PEG); 4. Commercial chicken diet containing 10% red grape pomace pre-treated with Viscozyme® - L (0.1% w/w) (ENZ); and 5. Commercial chicken diet containing 10% GP pre-treated with polyethylene glycol (5% w/w) and Viscozyme® - L (0.1% w/w) (PENZ) and were fed to four hundred Cobb 500 broiler chickens. The diets were randomly allocated to the pens and thus were replicated 8 times with each pen carrying 10 chickens. Weekly feed intake and body weights were recorded and used to calculate weight gain (WG) and feed conversion ratio (FCR). Blood was collected from brachial vein at 40 days of age for analysis of haematological and serum biochemical parameters.

Results revealed that the inclusion of 10% GP treated with PEG resulted in chickens with similar HCW as those on the conventional commercial diet while untreated GP reduced weight gain when compared to the CON diet. This is irrefutable evidence that tannins in GP reduce growth performance in Cobb 500 broiler chickens. Results revealed that GP inclusion in commercial broiler diets did not alter the physiological state of chickens; this reflects that GP is a potential feed ingredient. However, precautions need to be taken when high amounts of grape pomace are included in broiler diets.

5.2 Conclusions and Recommendations

The results from the feeding trial showed that grape pomace (*Vitis vinifera var. Shiraz*) inclusion levels at 5.5 % and 7.5 % promoted higher FCR compared to the commercial control diet. However, inclusion levels beyond 7.5 % can be achieved by pre- treating GP with polyethylene glycol and Viscozyme® - L to ameliorate the antinutritional effects of fibre and condensed tannins. Subsequent studies were conducted using polyethylene glycol and Viscozyme® - L to improve the utilization of GP in broilers to allow its inclusion at higher levels. The use of PEG to treat 10% GP resulted in chickens with similar HCW as those on the conventional commercial diet. On the other hand, untreated GP reduced weight gain when compared to CON diets; indicating that tannins and fibre in GP reduce growth performance. The treated GP had similar overall WG as commercial broiler diet suggesting that the antinutritional effects of tannins and fibre were successfully ameliorated. Furthermore, GP has the potential to improve food and nutrition security by boosting feed utilization efficiency while providing health benefits to consumers of poultry products.

5.3 Future research

Additional research is required to identify and evaluate other, possibly less-expensive strategies that can ameliorate the anti-nutritional effects of fibre and condensed tannins in order to improve the feed value of grape pomace for all birds of economic importance to man.

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6 LIST OF APPENDICES

APPENDIX 6.1. Ethics Certificate

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Research Ethics Regulatory Committee
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ETHICAL CLEARANCE LETTER OF STUDY

Based on approval by Animal Research Ethics Committee, Mafikeng Campus on 12/04/2018 after being reviewed at the meeting held on 12/04/2018, the North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby approves your study as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Enhancing the feed value of red grape (vitis vinifera var. Shiraz) pomace for broilers using peg and feed enzymes.

Study Leader/Supervisor: Prof V Mlambo
Student: C Ramanda

Ethics number: NWU-01239-18-A5

Application Type: NA
Commencement date: 2018-04-12 Expiry date: 2020-12-31 Category: 1

Special conditions of the approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the AnimCare. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreement incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-RERC via AnimCare:
  - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
  - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the AnimCare, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is forthwithly and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-RERC and AnimCare retains the right to:
  - request access to any information or data at any time during the course or after completion of the study;
  - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
    - any unethical principles or practices of the study are revealed or suspected,
    - it becomes apparent that any relevant information was withheld from the AnimCare or that information has been false or misrepresented,
    - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
    - new institutional rules, national legislation or international conventions deem it necessary.
- AnimCare can be contacted for further information or any report templates via Ethics.AnimCare@nwu.ac.za or 018 299 2197.

The RERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the RERC or AnimCare for any further enquiries or requests for assistance.

APPENDIX 6.2. Language Editing Certificate

DEPARTMENT OF BOTANY

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Cell: +27 72 626 8446 E-mail: Oziniel.Ruzvidzo@nwu.ac.za

Date: 29th May, 2019

To Whom It May Concern,

REF: Language Editing and Proof-reading of Dissertations/Theses

Dear Sir or Madam,

This serves to confirm that I have proof-read and edited the PhD thesis of **C Kumanda (28199642; Orcid.org/0000-0002-3370-2231)** entitled: **Enhancing the feed value of red grape (*Vitis vinifera var. shiraz*) pomace for broiler chickens using polyethylene glycol and enzyme.** The candidate then later corrected all the identified language and technical errors to my and the supervisor's utmost satisfaction. Thus the document presented here is of sufficient and acceptable academic standards.

Editor

Prof. O Ruzvidzo

Supervisor

Prof. V Mlambo

APPENDIX 6.3. Publication in Sustainability

sustainability

MDPI

Article

From Landfills to the Dinner Table: Red Grape Pomace Waste as a Nutraceutical for Broiler Chickens

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Abstract: The disposal of red grape pomace (GP) in landfills and by incineration has negative impacts on the environment. It is, therefore, imperative that alternative and sustainable ways of managing this waste product are identified. Using GP as a source of nutrients and beneficial bioactive compounds in avian diets is a potential waste-reduction and valorization strategy that promotes sustainable agriculture. However, there is limited information on the valorization of GP for this purpose. This study, therefore, investigated the effect of dietary inclusion of GP on growth performance, blood parameters, carcass characteristics, and breast meat quality traits of broilers. Four hundred, two-week old Cobb 500 broilers (279.2 ± 18.87 g) were allocated to 40 pens. Five isonitrogenous and isoenergetic diets were formulated by including GP in commercial broiler diets at 0 (GP0), 2.5 (GP25), 4.5 (GP45); 5.5% (GP55); and 7.5% (GP75). Feed intake, weight gain, feed utilization efficiency, hematology, serum biochemistry, carcass characteristics, and breast meat quality traits were measured. Chickens on GP75 had the least feed intake ($p < 0.05$) but there were no dietary effects on weight gain. Birds on GP0 had the highest ($p < 0.05$) feed conversion ratio (1.79) while those fed GP75 had the lowest ($p < 0.05$) ratio (1.45). Breast meat from broilers offered GP75 had the highest ($p < 0.05$) redness value (0.75) while the GP0 diet promoted the least ($p < 0.05$) redness value (0.49). Broilers fed GP55 and GP75 diets had higher ($p < 0.05$) feed conversion efficiency compared to GP0 birds. Inclusion of GP in broiler diets has the potential to reduce feed costs, thus making this valorization strategy a sustainable alternative to current pomace disposal methods. Adoption of this waste-reduction and valorization strategy promotes sustainable agriculture by contributing to food security and environmental stewardship.

Keywords: broiler diets; blood parameters; red grape waste; growth performance; meat quality; disposal methods

1. Introduction

Conservative estimates suggest that a third of all food produced ends up in landfills or incinerators, which exacerbates an already over-extended waste disposal system [1]. Reduction and valorization of food chain supply waste is critical in order to reduce the number of people that are food insecure. Indeed, most countries have committed to halving food waste by 2030 in recognition of threats to achieving sustainable development goals of food security, environmental protection, and energy efficiency caused by first-generation waste disposal methods [2]. Some of the sustainable valorization strategies for agro-waste include use as animal feed, animal bedding, organic fertilizers, heat, and cooking fuel [2]. When used as animal feed or organic fertilizers, agro-wastes make a direct contribution

APPENDIX 6.4. Publication in Animals

animals

MDPI

Article

Valorization of Red Grape Pomace Waste Using Polyethylene Glycol and Fibrolytic Enzymes: Physiological and Meat Quality Responses in Broilers

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Simple Summary: Red grape pomace (GP) waste, although rich in beneficial phenolic compounds, is traditionally disposed in landfills and through incineration, resulting in environmental pollution. The revalorization of GP as a source of nutrients and bioactive compounds in chicken diets is an environmentally sustainable and lower-cost alternative to current disposal methods. This approach has the potential to improve food and nutrition security while providing health benefits to consumers of poultry products. Unfortunately, the amount of GP that can be included in broiler diets is limited by fiber and condensed tannins found in this agro-waste. These compounds reduce the digestibility of GP in chickens, resulting in poor bioavailability of the beneficial bioactive compounds. Strategies are, therefore, required to ameliorate the effects of fiber and condensed tannins. This study investigated whether pre-treating GP with polyethylene glycol (PEG) and a cellulolytic enzyme mixture (Viscozyme®) would improve feed intake, physiological parameters, carcass characteristics and meat quality parameters of broilers. It was concluded that PEG treatment successfully ameliorated the anti-nutritional effects of condensed tannins. However, the cellulolytic enzyme treatment was ineffective against GP fiber.

Abstract: The amount of grape pomace (GP) waste that can be included as a functional feed in broiler diets is limited by anti-nutritional compounds such as fiber and condensed tannins. This study evaluated the effect of pre-treating GP with polyethylene glycol (PEG) and a cellulolytic enzyme mixture on physiological and meat quality parameters of broilers. Cobb 500 broilers (249.2 ± 20.31 g live-weight) were reared on five isoenergetic and isonitrogenous diets: 1. Commercial chicken diet (CON); 2. CON containing untreated GP at 100 g/kg (dGP); 3. CON containing 100 g/kg GP pre-treated with PEG (50 g/kg) (dPEG); 4. CON containing 100 g/kg GP pre-treated with enzyme (1 g/kg) (ENZ); and 5. CON containing 100 g/kg GP pre-treated with PEG (50 g/kg) and enzyme (1 g/kg) (PENZ). Overall body weight gains were similar in broilers reared on the CON, dPEG, ENZ and PENZ diets but lower in dGP chickens. The meat of birds reared on dPEG, ENZ, dGP and CON had a similar water-holding capacity, which was lower than in PENZ chickens. Diets influenced the size of duodenum, ileum, jejunum and caeca. Polyethylene glycol treatment promoted similar body weight gains and hot carcass weights as the commercial control diet, suggesting that the anti-nutritional effects of condensed tannins were successfully ameliorated.

Keywords: blood parameter; broiler; growth performance; meat quality; polyethylene glycol; red grape pomace waste