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Effect of air-classified faba bean protein on feed production, physical pellet quality, performance, and protein digestibility in broiler chickens

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Feed Manufacturing Technology

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ABSTRACT

Faba bean seed is a good source of crude protein (CP) (293.06 g/kg DM), however soybean meal (SBM) is the dominant protein source in broiler chicken diets. With air classification technique, faba bean seeds can separate to starch and protein rich fractions. Faba bean protein (FBP) (air-classified) may be a superior protein source compared to SBM for broiler chickens. This study was conducted to compare SBM and FBP in broiler chickens fed with both pelleted and extruded diets. After air classification, the CP content of FBP was 632.41 g/kg DM. Pelleting and extrusion processes were used to produce the SBM and FBP based diets. Physical feed quality was analyzed by hardness, pellet durability index (PDI), particle size distribution, expansion rate, and water stability. The broiler chicken performances (body weight, feed intake, and feed conversion ratio) were lower in the group fed FBP diets compared to the SBM fed group. The poor performance could be caused by anti-nutritional factors (especially heat-stabile NSP and RFOs). The protein digestibility was analyzed in the digesta content of the upper jejunum, lower jejunum, upper ileum, and lower ileum. FBP extruded (0.902) had the highest protein digestibility followed by FBP pelleted (0.875), SBM extruded (0.824), and SBM pelleted (0.813). The limitation of FBP diets on broiler chicken performances should be investigated closely.

Keywords: Faba bean, legume, anti-nutritional factors, protein digestibility, air classification, faba bean protein, crude protein, soybean meal, extrusion, pelleting.

CONTENTS

1. INTRODUCTION

Soybean meal is one of the most used plant based protein source in poultry in Europe because of its well-balanced amino acid (AA) composition and high digestibility. However, the SBM price has increased rapidly, making South American countries and the United States of America the main soybean producers and exporters in the world (Hartman et al., 2011). For a more stable price and accessible protein sources for European countries, legume seeds such as faba beans (*Vicia faba L.)* could become a competitor to soybean. According to Wiryawan and Dingle (1999), faba beans, chickpeas, lentils, and lupins could potentially substitute SBM. The main advantages of faba bean rather than other legume seeds (pea, chickpea, lentil, and lupin) are the nitrogen fixation from air and higher yield (Strydhorst et al., 2008). Additionally, faba beans are rich in protein and carbohydrates and are suitable for broiler chicken diets (Wiryawan et al., 1995). However, anti-nutritional factors (ANFs) such as protease inhibitors, oligosaccharides, non-starch polysaccharides (NSP), tannins, lectins, and phytic acid are found in faba bean as in most legume seeds. ANFs can negatively affect animal performance, nutrition utilization, and even animal health (Gatel, 1994). To eliminate the negative effects of ANFs, the mechanical and thermal processes such as air classification, pellet press, and extrusion are promising methods to utilize faba bean in broiler chicken diets. Faba bean seeds can be separated into protein rich and starch rich fractions by air classification. Faba bean protein fraction may then become a more competitive ingredient than SBM in the European market. Via air classification, it is possible to reduce tannins and NSP in the hulls by dehulling. By air classification, well adapted European climate and high yield faba bean crops can be a substitute for SBM in a broiler diet. Broiler chickens generally consume a pelleted feed because a pelleted feed increases feed intake, hygiene, and digestibility. (Svihus and Zimonja, 2011). Pellet press is the most common process for broiler chickens. Another thermomechanical feed process to produce animal feed is the extrusion technology. However, the extrusion process is usually used to produce aqua feed and

pet animal feed. According to Alonso (2000), the extrusion process increases nutrient digestibility and decreases the effects of ANFs such as protease inhibitors, lectins, phytic acid, and tannins.

In previous studies, faba bean diets were compared with SBM, other legume seeds, and between faba bean cultivations in broiler chicken diets (Moschini et al., 2005, Nalle et al., 2010, O'Neill et al., 2012, Woyengo and Nyachoti, 2012). Nutrient digestibility in extruded faba bean was studied in broiler chickens by Hejdysz (2016) and Diaz (2006). A search of the literature did not reveal any published study which evaluated air-classified faba bean protein fraction in broiler chickens. The most relevant research found was about grower pigs fed with pea and faba bean protein fraction (Gunawardena et al., 2010).

The aim of this study was to compare SBM and faba bean protein fraction (air-classified) in broiler chickens fed with both pelleted and extruded diets. The hypothesis of this study is that the protein digestibility of a pelleted-SBM based diet is expected to be higher than a pelleted faba bean protein fraction-based diet. Further, the protein digestibility of an extruded faba bean protein fraction-based diet will be improved via extrusion by eliminating the negative effects of ANFs. Broiler chicken performance, physical quality of feed, protein digestibility of faba bean protein fraction and SBM on pelleting and extrusion process was tested. To examine this hypothesis, the effect of air classification on faba bean, chemical changes on protein during pelleting and extrusion process, and protein digestion in broiler chickens were determined by chemical and physical analysis.

2. LITERATURE REVIEW

2.1. Legume seeds

Legume seeds are members of the *Fabaceae* or *Leguminosae* and the most well-known legumes are beans, pea, soybean, lentil, peanut, faba beans, lupine, chickpea, and peanut. They

are characterized by their fruits or pods. Legume seeds are one of the major plant protein sources for both animal and human consumption (Warsame et al., 2018). As an important source of plant proteins, legume seeds have provided protein to the increasing human population and they are also a good source of protein for farm animals such as cattle, poultry, swine, horses, and fish. Legume seeds are one of the few plant families that can fix nitrogen from air and therefore the root of legume plants are a source of nitrogen or protein (De and Antonio, 2015). They have a symbiotic relationship with *Rhizobia* bacteria which are found on the root nodules of their root systems (De and Antonio, 2015). *Rhizobia* can convert nitrogen (N_2) in the atmosphere to ammonia (NH_3) (De and Antonio, 2015). Some soil bacteria can convert ammonia to ammonium (NH_4) (De and Antonio, 2015). Ammonia is an available source of nitrogen for legume seeds. Nitrogen is used to form AAs and those AAs are then used for producing and storing protein in the seeds (De and Antonio, 2015). Consequently, legume seeds are rich in protein. Legume plants have the ability to serve as fertilizer. The remaining parts of legumes in/on soil can convert absorbed nitrogen into AAs. Those AAs are released into the soil and they are converted to nitrate (NO_3) that can serve as fertilizer for the next crop of plants (De and Antonio, 2015).

Legume seeds have a very important role in the diet of farmed animals because of their protein content. Legume seeds also contain carbohydrates (mainly starch), soluble fibers, minerals, and some vitamins. Furthermore, legume plants commonly synthesize a range of secondary metabolites (anti-nutritional factors) as a part of their protection against attack by herbivores, insects, and pathogens or as a means to survive in adverse growing conditions (Gatel, 1994, Khokhar and Apenten, 2003). The main ANFs in legume seeds are protease inhibitors, tannin, NSP, oligosaccharides, phytic acid, and lectins (Gatel, 1994). Legume seeds in monogastric animals' diets have become popular recently because of unstable SBM prices and the ban on meat and bone meal in Europe. Due to these conditions, the protein value of legume seeds has increased as a promising feed ingredient especially for broiler diets.

Legume seeds are known as protein rich ingredients. However, carbohydrates, crude fat, and ash content of legume seeds can vary between different types of legume seeds (Derbyshire et al., 1976). In Table 1, crude protein, carbohydrate, crude fat, and ash content are shown for different legume seeds.

Table 1. Some legume seeds' crude protein, carbohydrate, crude fat, and ash contents

Legume seed	Variety	Composition (g/100 g of sample)					Reference
		Protein	Fat	Fiber	Ash	Carbohydrate	
Faba bean	Shambat 616	26.6	0.70	5.75	3.30	57.1	(Elsheikh and Elzidany 1997)
	(Vicia faba L.)	29.9	0.94	N.D*	3.20	66.95	(Güzel, Sayar et al. 2012)
Bean	Kidney	23.58	0.83	24.9	3.83	60.01	USDA, (Boye et al. , 2010)
	V.C 2010	26.40	1.75	6.15	4.50	61.20	El-Adawy et al. (2003)
Chickpea	Garbanzo beans	19.30	6.04	17.4	2.48	60.65	USDA, (Boye et al. , 2010)
Lentil	Giza 9	31.4	1.15	6.75	4.16	56.53	El-Adawy et al. (2003)

*N.D: Not determined.

AAs have multiple functions such as nitrogen storage, building blocks of proteins, formation of glucose, production of hormones, neurotransmitter, etc. in animals' body (Wu, 2009). There are 20 main AAs which are seen in genetic code. Those 20 AAs are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine (Wu, 2009). However, in the diet of farm animals, only essential AAs are required because they cannot synthesize the essential AAs in their body, and therefore all essential AAs should be supplied in the diet by feed. Legume seeds are an important source of plant protein, and therefore the AA composition of legume seeds is high but unbalanced in essential AAs (Table 2). They contain high amounts of arginine, aspartic acid, glutamic acid, leucine, and lysine. On the other hand, the sulfur-containing amino acids (SAA) such as methionine and cysteine are low in legume seeds (Boye et al., 2010). Legume seeds cannot supply balanced essential AAs for monogastric animals

by themselves. Therefore, legume seeds should be mixed with cereal grain or synthetic methionine to obtain balanced essential AA composition.

Table 2. The amino acids composition of faba bean (*Vicia faba L.*), pea (*Pisum sativum L.*), and kidney bean (*Phaseolus vulgaris L.*).

Amino Acid	Faba bean (Vicia faba L.)		Pea (Pisum sativum L.)		Kidney bean (Phaseolus vulgaris L.)	
	PGG Tic[d]	South Tic[d]	Canadian pea[e]	Egyptian pea[e]	Canadian kidney bean[e]	Egyptian kidney bean[e]
Indispensable						
Arginine	25.0	25.0	7.93	8.31	5.83	5.45
Histidine	7.01	6.84	2.33	2.44	2.78	2.59
Isoleucine	9.55	9.72	3.89	3.09	4.15	3.42
Leucine	17.06	18.1	7.84	7,13	8.18	7,98
Lysine	14.4	15.0	6.25	6.39	6.05	5.32
Methionine	2.26	2.21	1.60[a]	0.90[a]	1.37[a]	1.76[a]
Phenylalanine	9.61	9.71	5.17	4.73	5.66	5.71
Threonine	7.51	8.13	4.46	4.15	4.48	4.72
Tryptophan	ND*	ND*	0.61	0.86	1.11	1.18
Valine	10.9	10.8	5.11	4.68	5.13	5.12
Dispensable						
Alanine	10.5	10.7	4.83	5.20	4.38	4.89
Aspartic acid	26.2	27.9	11.16[b]	12.37[b]	12.58[b]	12.94[b]
Cystine	3.86	3.71	0.35	0.59	0.00	0.24
Glycine	10.2	10.2	4.82	5.27	4.25	4.55
Glutamic acid	40.0	40.3	18.46[c]	18.03[c]	17.06[c]	16.88[c]
Proline	8.79	8.68	4.64	4.21	6.49	5.87
Serine	9.16	9.40	5.71	5.65	6,33	6.90
Tyrosine	7.78	7.98	3.34	3.17	2.75	2.95

[a] Methionine + Cysteine.
[b] Aspartic acid + Asparagine.
[c] Glutamic acid + Glutamine.
[d] Unit: g kg−1 dry matter (Nalle et al., 2010)
[e] Unit: g/100 g protein (Khattab et al., 2009)
*N.D: Not determined.

The most important nutrient in legume seeds is protein for this study. The average percentage of protein in legume seeds is 20 – 27% of dry matter (Gupta et al., 2010). The major proteins found in legume seeds are globulins and albumins (Horstmann et al., 1999). Albumins are water

soluble proteins and have molecular masses ranging between 5000 and 80,000 Da, representing 10 – 20% of legume protein (Karaca et al., 2011). They also contain enzymatic proteins, lectins, protease inhibitors, and amylase inhibitors. Globulins are stored in specialized inclusions in the cotyledon cells of legume seeds (Horstmann et al., 1999). They act as a reserve of raw material that can be readily and efficiently mobilized to aid the initial growth of the seed and seedling. The storage proteins serve only this purpose and have no enzymatic function (Utsumi et al., 1997). Globulins represent around 70% of legume protein and are salt soluble proteins. The major globulins found in legume seeds are legumin (11S) and vicilin (7S) (Karaca et al., 2011). Legumins have hexameric quaternary structures with an acidic subunit of 40,000 Da of molecular mass and a basic subunit of 20,000 Da of molecular mass. Vicilins have a trimeric structure with molecular masses of 175,000–180,000 Da. (Schwenke, 2001, Boye et al., 2010). Convicilin is the third storage protein in legume seeds and a 7S globulin. It has a different AA profile and contains very little carbohydrate and has a subunit molecular mass of 71,000 Da and a molecular mass in its native form of 290,000 Da including an N-terminal extension apart from vicilin protein. One of the distinctive features of convicilin, is that it contains sulphur containing AAs. Vicilin on the other hand lacks SAA. Glutelins and prolamins are minor proteins in legume seeds. Glutelins are soluble in dilute acid or alkali detergents and contain a higher amount of SAA (such as methionine and cystine) than the globulin protein (Osborne, 1924). Prolamins are alcohol soluble and rich in proline and glutamine AA (Boye et al., 2010). According to Young and Pellett (1994), the nutritional quality of proteins can be defined by the composition of essential AAs and its digestibility. The essential AA content in legume seeds are of critical importance as a valuable animal feed ingredient. The ratio between albumin: globulin and legumin: vicilin are determined by the majority of AAs in legume seeds. The AA composition of legume seeds is usually high in lysine, leucine, aspartic acid, glutamic acid and arginine, but low in methionine, cysteine and tryptophan (Swanson, 1990). Before the utilization of AAs (proteins), they need to be digested by protease enzymes. However, legume seeds also contain protease enzyme inhibitors such as

trypsin and chymotrypsin inhibitors (Gatel, 1994). Those inhibitors bind the protease enzymes and decrease protein digestibility. For legume seeds, processing such as pellet pressing or extrusion may reduce or inactivate the protease inhibitors from feed (Alonso et al., 2000). Otherwise, protein digestibility of feedstuff may be dramatically reduced without proper processing. There is a demand for the ingredients with higher protein digestibility because they have a higher nutritional value especially for monogastric animals. According to Marquez and Lajolo (1990), the isolated globulin from *Phaseolus vulgaris* had 89.5% protein digestibility and isolated albumin had protein digestibility of 79.1% however, the protein digestibility of the gluten isolate was 73.2%.

2.2. Faba bean seeds

Faba bean (*Vicia faba L*.) originated in the Near East (Iraq, Iran, Syria, and Turkey) in the 10th millennium. It is the oldest legume seed used in agricultural practices (Cubero, 1974). *Vicia faba L*. is known as field bean, horse bean, bell bean, English bean, and broad bean around the world. The largest producers of *Vicia faba* are China (33%), Ethiopia (18%), the United Kingdom (14%), Australia (9%), France (3%), and Germany (2%). Along with these countries, *Vicia faba L.* is planted in around 70 countries. The total production of faba bean is around 4 million tons annually and 2.2 million hectares of land is used to plant faba bean (Warsame et al., 2018). According to current data, it might be one of the highest yielding and efficient legume seed (Warsame et al., 2018). According to Watson (2017), faba beans can fix nitrogen better than pea and soybean combined in the same area, therefore planting faba beans decreases synthetic the usage of fertilizer. *Vicia faba* is thus a more sustainable crop to plant (Warsame et al., 2018).

Faba bean has a great potential to supply protein rich ingredients for monogastric animals. Besides protein, they also contain starch, fiber, vitamin, and minerals (Table 1). Containing desirable amounts of different nutrients makes faba bean seeds a good ingredient for animal feed

and food for humans. The protein content of faba bean seeds can vary considerably between different genotypes and it can be 19%-39% protein of dry matter (Warsame et al., 2018).

Table 3: Nutritional content of different cultivations of faba bean.

	Vicia faba cultivars						
	Merlin[a]	Olga[a]	Taifun[a]	High tannin faba beans[ad]	Low tannin faba beans[ad]	Faba bean[b]	Lielplatone[c]
Crude protein	296	314	283.3	310	319	259	29.4
Starch	438	394	N.D	412	427	327.2	44.5
Fat	11	12	18.6	19	20	16.1	1.6
Crude fiber	N.D	N.D	81.3	99	88	77.7	6.5
Ash	N.D	N.D	57.3	N.D	N.D	33.8	3.4
Sugar	N.D	N.D	N.D	38	44	40.3	N.D
Reference	(Hejdysz et al., 2016)	(Hejdysz et al., 2016)	(Witten et al., 2018)	(Duc et al., 1999)	(Duc et al., 1999)	(Diaz et al., 2006)	(Proskina et al., 2017)

[a] g/kg dry matter.
[b] (g/kg as fed basis).
[c] (% DM).
[d] Means of four low-tannin lines carrying gene zt1, compared to the mean of their four high-tannin isogenics.

Faba bean seed is high in nonessential AAs and low in essential AAs as are most legume seeds in Figure 1. Around 45% of the AAs in faba bean consist of glutamic acid, aspartic acid, arginine, and leucine. However, faba bean is high in lysine which is one of the most required essential AAs for monogastric animals. On the other hand, the SAAs such as methionine, cysteine, and tryptophan are poor in a faba bean seed. The SAA, especially methionine, are the first limiting essential AAs after lysine. The level of essential AAs in faba beans are not optimal for monogastric animals.

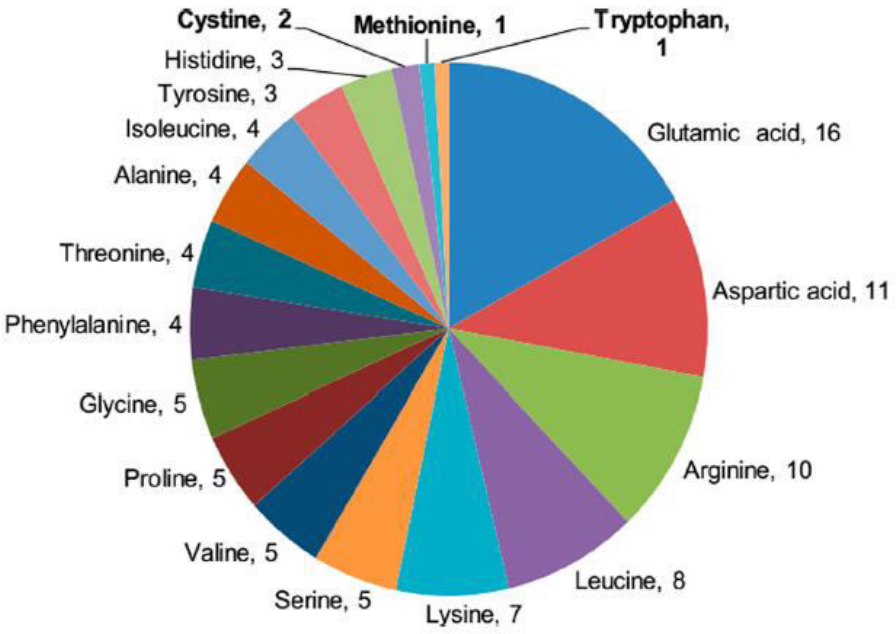


Figure 1. Amino acid composition (g/16 g N) of *Vicia faba* seed protein (Warsame et al., 2018).

Globulins and albumins are the major classes of storage proteins in faba bean (*Vicia faba L.*). Globulins are enzymatically inactive proteins stored in seed cotyledons and their main mission is to supply the nutrients needed for seed germination and seedling growth and development. Globulins form approximately 80% of total seed protein in faba bean legumin (11S) and vicilin (7S) are the main globulin sedimentation (Horstmann et al., 1999). Legumin constitutes more than 50% of faba bean globulins and it is a hexameric shaped (Horstmann et al., 1999). There are two major subunits calling legumin A and legumin B. Both of their polypeptides are similar (Warsame et al., 2018). However, legumin A contains more methionine residues which is the most notable difference between two subunits (Warsame et al., 2018). The other globulin proteins are vicilin and convicilin which have trimeric protein shapes. On the other hand, albumins are mainly enzymatically active proteins and anti-nutritional agents such as protease inhibitors, amylase inhibitors, lectins, etc. One of the main limiting factors in faba bean proteins is the lack of SAA. According to Warsame (2018), there is a correct proportion between SAA and sulfur-containing

proteins. To obtain more SAA in faba bean seeds, first sulfur-containing proteins should be increased in faba bean seeds. In globulins, vicilin is almost absent of SAA. However, convicilin contains SAA. Legumins contain higher SAA because of legumin A subunit that contains methionine and cysteine. Beside globulins, albumins contains higher SAA. Elongation factor Tu, citrate synthase, albumin 2, defensins 1 and 2, and Bowman−Birk inhibitors are examples of some albumin proteins (Liu et al., 2017, Jackson et al., 1969).

2.3. Anti-nutritional factors in faba bean seeds

Anti-nutritional factors in faba bean seeds can have negative effects in animal and human metabolism. The main ANFs in *Vicia faba* are protein inhibitors, lectins, phytic acid, tannins, oligosaccharides, and NSP. Those ANFs have mainly adverse effects on protein digestion and absorption but they may also have negative effects on carbohydrate digestion, fat digestion, mineral utilization and vitamin availability (Huisman et al., 1989).

2.3.1. Protease inhibitors

Protease inhibitors inhibit the activity of the enzyme trypsin and chymotrypsin in the animals' system. Protein inhibitors are generally connected with albumin protein fractions and they are low molecular proteins and polypeptides in molecular mass between 8 – 30 kDa. As albumin protein, most of the protein inhibitors are water and acid soluble. (Visitpanich et al., 1985). The most common protease inhibitors in legume seeds and soybean are Kunitz and Bowman-Birk type trypsin inhibitor. The Kunitz inhibitor consists of single-chain polypeptides with two disulfide bridges and it can block only the trypsin enzyme. Heat treatment inactives the Kunitz trypsin inhibitor and gastric juice may also inactivate the Kunitz trypsin inhibitor (Guillamon et al., 2008). Bowman–Birk type inhibitors are formed as single-chain polypeptides with seven disulfide

bridges. Trypsin and chymotrypsin enzymes are blocked by Bowman–Birk type inhibitors. Because of the number of disulfide bridges, Bowman–Birk inhibitors are more resistance to heat and gastric juice than Kunitz type trypsin inhibitors (Guillamon et al., 2008, Gupta, 1987, Jukanti et al., 2012). Their heat and enzymatic resistance is related with number of disulfide bridges (Guillamon et al., 2008). When the number of disulfide bonds increase, the protease inhibitor becomes more compact and more resistance to heat and enzymes. Whereas, if the number of disulfide bonds decrease such as Kunitz trypsin inhibitors, heat treatment causes the protease inhibitor unfolded.

Protease inhibitors are rich in sulfur and SAA. Therefore, they are high in cysteine and methionine and they contain up to 15% cysteine of their AA composition (Lampart-Szczapa, 2001). The reason for that, is due to that sulfur is used to protect the seed against insects or microorganism. In compliance with Birk research in 1968, protease inhibitors do not inhibit endogenous proteins of the host plant. They inhibit only other organisms' protease enzymes (Birk, 1968). When farm animals or human consume raw legume seeds, protein digestion and utilization decreases because of the inhibition of trypsin and chymotrypsin enzymes. Pancreas then produce more proteolytic enzymes and its size becomes larger due to more enzyme production. If this condition becomes permanent, pancreas become shrinked and lose its main functions. The other functions of protease inhibitors (trypsin and chymotrypsin) and beta amylase inhibitors in legume seeds may have beneficial effects such as natural pesticides, therapeutic agents, and for inhibition carcinogenesis and HIV infection in plants (Rutstein et al., 1997).

Protease inhibitors have a negative effect on protein digestibility and utilization. However, it is possible to reduce protease inhibitors' activities. Trypsin and chymotrypsin inhibitors can be resistant to the heat itself. Yet, temperature, moisture, duration of heating, and particle size distribution can be effective to decrease or even remove inhibitor's activity (Rackis et al., 1974). According to Sitren (1985), the raw soybean inhibits the maximum 76% of the trypsin enzyme,

the dry-heated soybean inhibits 61% of trypsin enzyme, and the moist-heated soybean inhibits 11% of trypsin enzyme in animal (rat) experiment. Trypsin inhibitors activity decreases 15 unit when heat applied to the raw soybean. There was 65 unit decreases in trypsin inhibitors activity when heat and moisture was applied to the raw soybean. Heat and moisture together reduce the activity of trypsin inhibitors. Extrusion is a high temperature and pressure in short time process. It can remove 33-98.9% of trypsin and 52.8% of chymotrypsin inhibitors in faba bean (Hejdysz et al., 2016, Alonso et al., 2000) . It is possible to remove all trypsin inhibitor in legume seeds. For example, steaming for 60 minutes (min) or autoclaving of soybean seed for 30 min completely inactivated the inhibitor activity (Kapoor and Gupta, 1978). Cooking, microwave treatment, pressure cooking, toasting, soaking, germination, and chemical treatments are other processes that reduce trypsin inhibitor activity in legume seeds (Akande and Fabiyi, 2010). As a result, time or duration of heat is one the key point to remove trypsin inhibitor.

2.3.2. Lectins

Lectins are also named phytohaemagglutinins. They are proteins and glycoproteins. Lectins have ability to bind carbohydrate, calcium, and a transition metal ion and identify by their diverse sugar structures. Lectins can be found both albumins and globulin proteins in legume seeds. Legume lectins contain 1-10% of total protein in legume seeds and lectins contain approximately 4-10% carbohydrates (Lampart-Szczapa, 2001). Lectins are founded in plants, animals, bacteria, and viruses. There are more than 200 three dimensional structures of lectins and more than half of them belongs to plant (legume) lectins (Vijayan and Chandra, 1999). Therefore, legume lectin structures are varied widely because of their protein and carbohydrate interaction. In legume seeds, lectins contribute to seed protection against bacteria and viruses. During bacteria or viruses invasion, legume lectins hold bacteria or viruses saccharides (sugar).

Legume lectins also adjust a symbiotic relationship with the bacterium Rhizobium by binding the root noodles and storing and transferring carbohydrates (Gupta, 1987, Lampart-Szczapa, 2001).

Legume lectins are characterized as ANFs for farm animals such as poultry, swine, fish, ruminant, and equine. First, there is no nutrient content in legume lectins because they are not digestible by farm animals. Second, by Pusztai (1989), legume lectins bind the mucosal surface of the digestive tract and degenerate epithelium morphology. This degeneration over time may cause malfunction, disruption, and lesion in the small intestine and inhibiting absorption of nutrients from the small intestine. Third, lectins may reduce the activity of glycoprotein enzymes in the digestive tract. Therefore, trypsin and chymotrypsin enzymes may not breakdown proteins to polypeptides and/or AAs efficiently (Gupta, 1987). Then, the physiology of pancreas is affected negatively by producing more protease enzymes. Forth, legume lectins cause hemolytic anemia by agglutinated red blood cells. There are production, health, or life consequences of feeding farm animals by lectins (Pusztai, 1989).

Lectins are founded in almost all legume seeds and they are an anti-nutritional agent for farm animals. The level of lectins can be reduced or removed from legume seeds. This event can be done with heat and moisture. Only heat could not inactivate lectins but a combination of heat and moisture could remove lectins and lectin residues (Jaffé and Vega Lette, 1968). According to Sitren (1985), the raw soybean contains 112.1 µg/g lectins and dry heated soybean contains 94.7 µg/g lectins. On the other hand, moist, heated soybean contains no lectin. This research showed that heat without moisture cannot remove more than 16% of the lectins. Extrusion cooking and autoclaving were completely inactivating the lectins, activity in feedstuff (Akande and Fabiyi, 2010). However, steam pressing (pellet press) could not reduce the lectin activity in *Vicia faba (Gatel, 1994)*.

2.3.3. Tannins

Tannins are ANF found in legume seeds and known as phenolic compounds. Tannins are located generally in the seed coat but they can also be found in cotyledon in legume seeds. Their molecular weight ranges from 500 Da to more than 3000 Da (Hassanpour et al., 2011). The reason for a large molecular weight range is that tannins can be complex with proteins, starch, cellulose, and minerals (Hassanpour et al., 2011). Tannins are water soluble components, but the higher molecular weight structure's tannins may not solubilize in water due to a complex structure. The purpose of the tannins in plants is to be a part of the defense system against insects, birds, and herbivores (Min et al., 2003). Tannins are divided into two groups by their chemical structure and properties such as hydrolysable and condensed tannins (Hassanpour et al., 2011). Hydrolysable tannins contains a carbohydrate which is generally D-glucose as a central core and the hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Haslam, 1989). Hydrolysable tannins are generally found in fruit seed, pod, and plant galls. However, the concentration of hydrolysable tannin is lower than condensed tannin. The breaking down products of hydrolysable tannins can be absorbed by the small intestine and might be toxic for the animal (Dollahite et al., 1962, Min et al., 2003). Condensed tannins are the most abundant type of tannins in plants. The physical and biological properties of condensed tannins are effected by chemical structure and their structure is formed by flavanoid units (flavan-3-ol) linked by carbon-carbon bonds (Hassanpour et al., 2011). The interaction between condensed tannin and protein consists of hydrophobic and hydrogen bonding. The pH is a key factor between condensed tannin and protein interaction because each protein has a distinctive pH optimum. According to Jones and Mangan (1977), condensed tannins are able to connect with proteins at pH 3.5–7.5 to create complex of condensed tannin-proteins. When the pH is less than 3.5, condensed tannin-proteins complex is released. Tannin-protein complexes can be found in both an insoluble and soluble form. Besides protein, tannins can form of complexes with cellulose, hemicellulose, pectin, nucleic acids, steroids, alkaloids, and saponins

(Hassanpour et al., 2011). The anti-nutritional effects of tannins is mainly caused by complexes with other nutrients. For instances, tannin-protein complexes can be indigestible by farm animals.

2.3.4. Phytic acid

Phytic acid is an ANF for monogastric animals and it is the major storage form of phosphorus in legumes, cereals, and oilseeds crops. Phytic acid in Figure 2 is a hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol and also known as inositol hexakisphosphate (IP6) or phytate (salt form) (Kumar et al., 2010). Insoluble phytate salt occurs when phytic acid forms with phosphorus, calcium, zinc, magnesium, iron, and copper. Molecular weight of phytic acid is 660 kDa (Singh, 2008). At neutral pH, the phosphate groups in phytic acid have one or two negatively charged oxygen atoms, hence cations can bind strongly between two phosphate groups or weakly with a single phosphate group (Singh, 2008). In legume seeds, phytate is mainly stored in the dicotyledons.

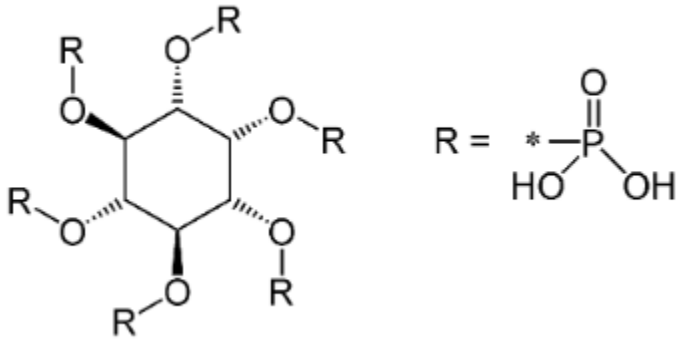


Figure 2. Chemical structure of phytic acid (Kumar et al., 2010).

Phytate salt occurs during the maturation of the legume seed and in the seed of legumes, phytate content is 60–90% of the total phosphate (Kumar et al., 2010). The main function of phytic acid or phytate in legume seeds is to store phosphate in the seed until the plant needs. Phytic acid or phytate has anti-nutritional effects on monogastric animals because it constitutes complexes with minerals (phosphorus, calcium, zinc, magnesium, iron, and copper), proteins, carbohydrate (starch), lipids, and protease enzymes (pepsin and trypsin). In this condition, those nutrients are unavailable or less available for monogastric animals because endogenous phytase enzymes are found in insufficient amounts in monogastric animal`s digestive system (Singh, 2008). In the diet for monogastric animals, phytic acid or phytate should be as low as possible or phytase enzyme should be added in the diet to avoid anti-nutritional effects of phytic acid. Phytic acid impacts negatively on protein digestion and absorption when it is in phytate salt form.

Phytate may create a complex with proteins and this complex is resistant to protease enzymes such as pepsin and trypsin. Phytate-protein complex depends on pH. According to Kumar (2010), phosphoric acid groups of phytate bind with the cationic group of basic AAs such as arginine, histidine, lysine, and forms binary protein–phytate complexes when the pH value is lower than the isoelectric point of proteins. The protein–phytate complexes are insoluble complexes above pH 3.5 and they become soluble only below pH 3.5. Therefore, the protein–phytate complexes can inhibit protease enzyme’s activities, protein digestion, absorption, and utilization. Phytate salt has negative impacts on starch utilization. A protein–phytate complex can bind starch with protein – starch interaction and phytate can bind starch molecules itself by hydrogen bonds (Rickard and Thompson, 1997). The phytate-starch complexes decrease digestibility and absorption of more insoluble complexes in digestion. Lipid is used as a secondary energy source in poultry diets. However, there may be a risk of energy waste when phytate and lipid are used together in the diet. Leeson (1993) observed that lipid and Ca phytate may form metallic soap in poultry digestive tract and Matyka (1990) also observed that when young chicks

were fed a lipid and phytate containing diet, phytate utilization was inhibited and a large percentage of fat was excreted as soap fatty acids. Therefore, high phytic acid and lipids in the poultry diet may cause large amount of unutilized fat source.

2.3.5. Non-starch polysaccharides

Non-starch polysaccharides (NSP) are included in dietary fiber with oligosaccharides and other free sugars. Legume seeds contain significant amounts of NSP. Gdala and Buraczewska (1996; 1997) found that the average of NSP in faba beans is 177 g/kg, pea is 185 g/kg, and in lupins is between 320 to 400 g/kg DM in different species. NPS are separated into three main groups that are cellulose, non-cellulosic polymers (arabinoxylans, mixed-linked β-glucans, mannans, and xyloglucan) and pectic polysaccharides (arabinan, galactan and arabinogalactan) (Bailey, 1973).

Cellulose is the most abundant plant polymer comprising over 50% of all the carbon vegetation worldwide (Sinha et al., 2011). It is a complex polysaccharide and consists of 3000 or more β -(1→4) linked D-glucose units with molecular weights of over 1,000,000 Da (Sinha et al., 2011). Cellulose is a straight-chain polymer where no coiling or branching occurs because of the equatorial conformation of the glucose residues and the molecule adopts an extended and rather stiff rod-like conformation (Sinha et al., 2011). Cellulose is found as a form of large micro fibrils. Thus, cellulose is highly insoluble in water, but it can swell in concentrated sodium hydroxide solutions (Sinha et al., 2011). Some bacteria and fungi are able to digest cellulose. However, cellulose is indigestible for monogastric animals because they cannot synthesis cellulase enzyme in their digestive tract. In ruminant animals, bacteria and fungi can digest cellulose in the rumen.

Arabinoxylans are found abundantly in cereals and grasses. They are generally composed mostly of two pentoses: arabinose, and xylose, and their molecular structure consists of a linear

a (1→4)-β-D-xylan backbone and β-xylopyranosyl unit of the xylan backbone are linked to α-L-arabinofuranose units as side branches (Sinha et al., 2011). Arabinoxylans are mostly insoluble in water as they have alkali-labile ester-like cross links rather than a simple physical entrapment in the cell walls (Mares and Stone, 1973). However, they can absorb about ten times their weight of water because they form highly viscous solutions in the cell wall. Arabinoxylans also form a gel network when with oxidative agents, such as water and peroxidase. The mixed-linked β-glucans are second type non-cellulosic polymers and members of the monocotyledon family *Poaceae* which are cereal grains. They consist of a linear chain of glucose units joined by both β-(1→3) and β-(1→4) linkages (Bengtsson et al., 1990). In the mixed-linked β-glucans, the average monomers are corresponding to degrees of polymerization of 1,200-1,850 and the average molecular masses reported for cereal-β-D glucans range from 200,000 to 300,000 (Woodward et al., 1983). The mixed-linked β-glucans and cellulose have similar physical properties. They both consist of β linked glucose units. However, cellulose is comprised only of β-(1→4) linkages and hence, cellulose is non-soluble, highly crystalline, and rigid. On the other hand, the mixed-linked β-glucans contain both β-(1→3) and β-(1→4) linkages. Because of the β-(1→3) linkages, the β-glucans molecules are more soluble and flexible than cellulose (Anderson and Bridges, 1993). Even the β-glucans are more soluble than cellulose, yet they cannot be digested in the small intestine by monogastric animals. There is, however, limited β-glucans digestion in the large intestine due to the activity of microorganisms. Mannans are non-cellulosic polymers and composed of the hemicellulose fraction in softwoods and branch out widely in plant tissues (Petkowicz et al., 2001). In plants, their main role is binding hemicellulose to cellulose and creating hardness in a cell basis (Liepman et al., 2007). Mannans constitute a β-1,4-linked backbone containing mannose or a combination of glucose and mannose residues (Liepman et al., 2007). In addition, the mannan backbone can be substituted with side chains of α-1,6-linked galactose residues. Mannans have been divided into four subfamilies: linear mannan, glucomannan, galactomannan, and galactoglucomanan (Petkowicz et al., 2001). Linear mannans are

homopolysaccharides composed of linear main chains of 1,4-linked β-D-mannopyranosyl residues and contain less than 5% galactose. Mannans are generally insoluble in water and highly dense (Petkowicz et al., 2001). The galactomannans are reserve polysaccharides in the seeds of the leguminous plants and are located in the endospermic part of the seeds (Dey, 1978). Galactomannans are composed of β-(1→4)-linked mannan chains with α-(1→6)-linked galactosyl side groups. They are water soluble and can absorb water, thus providing a water-holding function for the seed and play a crucial role to prevent the complete drying of the seeds that would lead to protein denaturation (Parvathy et al., 2005). Glucomannans have physical properties similar to those of cellulose and are therefore found in plant cell walls associated with celluloses. They store polysaccharides in the seeds. Many of these glucomannans are water soluble and are composed of a β-(1→4)-linked mannan chain with interspersed glucose residues in the main chain and are often acetylated (Popa and Spiridon, 1998). Both the solubility and the viscosity of the galactomannans are influenced by the mannose-to-galactose ratio, which can vary from 1 to 5 (Reid, 1985). Galactoglucomannans contain D-galactose residues attached to both D-glucosyl and D-mannosyl units as α-1,6-linked terminal branches (Popa and Spiridon, 1998). The presence of D-galactose side-chains render the galactoglucomannan to be soluble in water because it prevents the macromolecules from aligning themselves, thereby resulting in the formation of strong hydrogen bonds" (Sinha et al., 2011).

Neutral pectic polysaccharides are divided into the three main types; arabinans, galactans, and arabinogalactans. Pure forms of arabinans and galactans are found in low amounts in the plant cell wall. Arabinans are highly branched and consisting of a core of α-1, 5 arabinosyl residues containing α -1,3- and α -1,2-linked arabinosyl side chains. It accounts for 9% of the primary cell wall of dicotyledonous plants (Darvill et al., 1980). Galactans are mostly linear ß-1,4-linked D-galactose polymers with occasional single L-arabinose branches (Ghosh and Das, 1984). The arabinogalactans contain ß-1, 4-linked galactose chains carrying arabinose residues at the

3 and 6 positions which are further substituted. However, the arabinogalactans occur in two distinct types in plant cell walls. Type I is very common in grain legumes, and is characterized by β-(1→4) galactan backbone substituted with 5-linked and terminal arabinose residues (Cheetham and Wootton, 1993). The type II arabinogalactan is commonly found in the rapeseed cotyledon. Type II is characterized by β-(1→3,6)-linked galactose polymers associated with 3- or 5-linked arabinose residue (Siddiqui and Wood, 1972).

The anti-nutritional agents in NSP are generally related with solubility in monogastric animals. Knudsen and Hansen (1991) observed that pigs can digest almost all the soluble NSP, but they can only digest between 34-60% of insoluble NSP. The other monogastric farm animals such as chickens are less efficient at utilizing NSP than pigs. An adult chicken can degrade up to 80-90% of soluble NSP. However, there was no degradation observed on insoluble NSP (Carré et al., 1995). Carré (1995) also observed that adult cockerels digested NSP better than broiler chickens. NSP digestibility improves with aged due to the adaptation of microflora. Both studies concluded that pigs are better NSP digesters than chickens, and the microflora can adapt to dietary NSP over time and then improve NSP digestion significantly. Soluble NSP has more anti-nutritional effect than insoluble NSP in monogastric animals. Soluble fractions of NSP reduce digestion and absorption of nutrients. Poultry is affected by NSP more than swine (Choct et al., 2010). In a digestive system, soluble NSP increases the viscosity of the digesta, and the retention time of digesta also increases. In this condition, the small intestine becomes a good environment for fermentative microflora. The microbial fermentation occurs in the small intestine and the production of volatile fatty acids (VFA) increases the energy content of the feed. However, digestion and absorption of nutrients decrease and as a result, poor animal performance observed (Choct et al., 2010). Consequently, fermentative microflora degrades nutrients in the small intestine and produces mainly VFA, but the amount of absorbed nutrients in the small intestine

decreases. This situation may create unbalanced nutrient content for monogastric animals, especially poultry.

2.3.6. Oligosaccharides

Oligosaccharides are saccharide polymers containing between 3-10 monosaccharides. All oligosaccharides are not anti-nutritional agents for monogastric animals. For instance, sucrose is an oligosaccharide which is also digestible for monogastric animals. On the other hand, oligosaccharides are classified within the dietary fibers such as raffinose family of oligosaccharides (RFOs) (Figure 3) and it is an ANF for monogastric animals. They consist of linear chains of galactosyl residues, linked to the glucose moiety of sucrose via α - (1→6) glycosidic linkage and raffinose, stachyose, verbascose and ajugose are known the members of RFOs (Avigad and Dey, 1997, Peterbauer and Richter, 2001). Raffinose is generally found in monocotyledon seeds, dicotyledon seeds contain mainly stachyose and verbascose, and ajugose is found in a low amount in the seeds (Peterbauer and Richter, 2001).

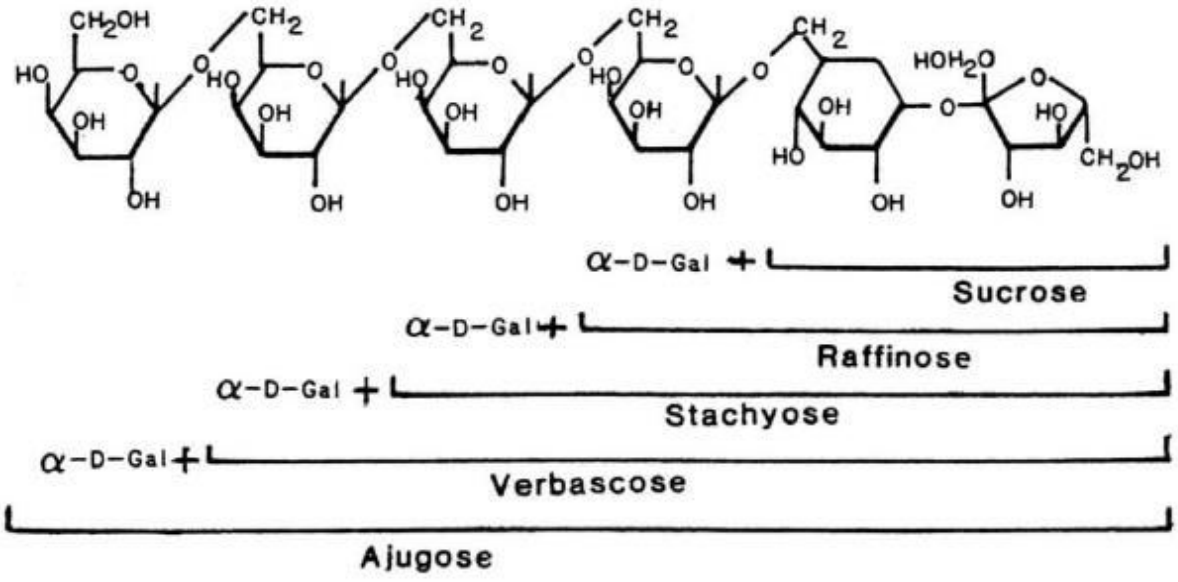


Figure 3. Chemical structure of the raffinose family of oligosaccharides (Choct et al., 2010).

The RFOs in legume seeds may be regarded as storage carbohydrates. They generally contain 2–10% of dry matter in the seeds (Horbowicz and Obendorf, 1994). They are broken down rapidly during the early stages of germination and may thus provide readily available energy and substrates to support growth (Peterbauer and Richter, 2001). Legume seeds are usually rich in raffinose family of oligosaccharides. For example, lupin species contain varies amounts of RFOs such as 60 g/kg DM in Lupinus angustifolius and 105 g/kg DM in *Lupinus luteus*. The average RFOs in faba beans is about 28 g/kg DM and peas contain 58 g/kg DM. Lupin seeds have stachyose as a higher content of RFOs, faba bean seeds contain verbascose, and pea seeds have both stachyose and verbascose as a dominated RFOs source (Gdala and Buraczewska, 1997, Gdala and Buraczewska, 1996).

Oligosaccharides in raffinose families referred to aslined ANFs for monogastric animals and humans because α-galactosidic linkages of RFOs are not digestible due to an endogenous enzyme deficiency in the small intestine. However, the microorganisms in the lower part of the digestive system can break down α-galactosidic linkages and the result of microbial fermentation creates short chain fatty acids, carbon dioxide, and hydrogen. This event is the reason for flatulence after consuming RFOs in the diet (Baucells et al., 2000). On the other hand, RFOs may act as a prebiotic in the lower part of the digestive tract because they may be degraded by specific bacteria such as lactic acid bacteria, resulting in a desirable gut microflora (Tortuero et al., 1997). The RFOs also increase hydrogen production, diarrhea, and retention time of digesta (Choct et al., 2010). According to Saini (1989), diarrhea may be observed in high amounts of legume seed-containing diets. The reason for the diarrhea is that the digestion of RFOs in the lower intestine may change the osmotic differences between the mucosa and plasma tissues. In poultry, oligosaccharides increase retention time of digesta content which also increases microbial fermentation which causes rapid hydrogen production. Diarrhea may occur and as a result, nutrient digestion and absorption reduces (Choct et al., 2010).

2.4. Air classification

Different types and designs of air classifications have been used to separate large from small particles or large from lower density materials for different industries such as food, cement, mining, and recycle industries (Shapiro and Galperin, 2005, Ferrari et al., 2009, Rowley, 2001). The air classification technology has been used in the animal feed industry for both research and commercial purposes. Centrifugal air classifiers, especially vortex air classifiers, were used to separate animal feed ingredients such as cereal grains, legume seeds, and oil seeds. This method separates light particles from heavy particles in finely grinded grain or legume flour by utilizing stream air. The light particles are the protein fraction whereas heavy particles are the starch fraction (Vose, 1978).

Fine grinding is essential for air classification and some ingredients such as legume seeds are dehulled before air classification. In the seed coat, the major part of the tannins and fibers are found in faba beans (*Vicia faba*, L. major). The dehulling is removing the seed coat (hull) from the seed (Vidal-Valverde et al., 1998). Dehulling can be used in legume seeds, oil seeds, and cereal grains and is not an essential process for air classification. However, some animal species, some animals of a certain age, or some products are required to not contain or contain the minimum amount of specific contents such as tannins, cellulose, hemicellulose, lignin, etc. In this condition, dehulling can be used. After the dehulling, the milling of material occurs. Different grinders are used by different researchers. For example, Gunawardena (2010) used a cracking mill (Ferrel-Ross, Bluffton, IN) and an Alpine Contraplex Wide Chamber Pin Mill (type A250, Alpine Aktiengesellschaft, Augsberg, Germany), De Santis (2015) grinded the dry raw materials with Sprout Matador hammer mill, and Coda (2015) milled dehulled faba bean by cutting mill (Retsch GmbH, Haan, Germany). Level of grinding is an important factor during air classification to separate proteins from starch granules. More than 95% of the grinded flour should be milled smaller than 100 μm (Gunawardena et al., 2010). After milling, air classification separates the

smaller protein-rich fragments from the larger starch granules by a rotor classifier. The finely grinded flour is distributed by stream air and enters from below and rises upward into a conical vessel containing a rotating classifier wheel with blades at the top. These blades create a centrifugal-counter flow separation zone in which the small and large particles are separated (Schutyser et al., 2015). In Figure 4, the steps of air classification is shown.

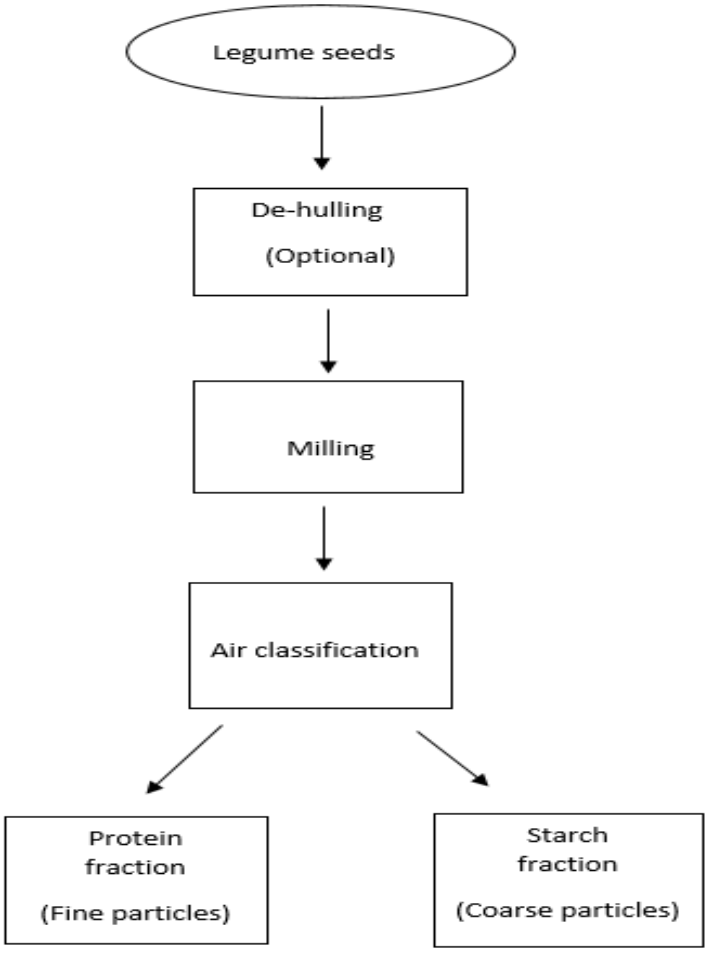


Figure 4. The steps of air classification process.

The cotyledon structures in legume seeds (*Vicia faba L.*) are similar to the endosperm of cereals (wheat, barley, etc.). The main nutritional contents such as proteins, starch, and lipids (for soybean and rapeseed) are closely collected in the seed (Owusu-Ansah and McCurdy, 1991).

According to Owusu-Ansah and McCurdy (1991), legume seeds and cereal grain tissue structure is similar. Therefore, wheat can be used as an example for legume seeds. The endosperm of wheat is built up from multiple cell types, which are packed with starch granules embedded in a protein matrix which are storage proteins and synthesized as protein bodies (Delcour and Hoseney, 2010). During the maturation of wheat, the protein bodies are compressed together into a matrix. In wheat, most of the starch granules are large and lens-shaped in 20 – 25 µm. On the other hand, a number of smaller spherical shaped starch granules of 2-10 µm are less than larger starch granules. After milling (pin mill) of wheat, there are wheat flour particles with a diameter below 40 µm. With air classification, starch fraction contains the largest starch granules of 10 – 40 µm and protein fraction contains protein matrix fragments and small starch granules of smaller than 10 µm (Figure 5) (Schutyser and Van der Goot, 2011).

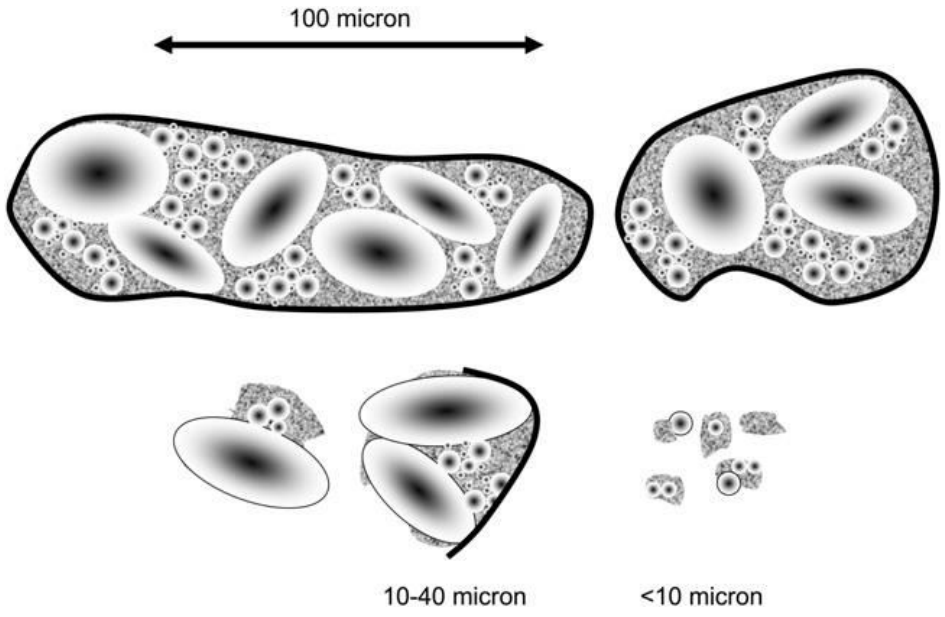


Figure 5. Schematic drawing of typical cells present in the endosperm of wheat and related fragments containing high starch (10 - 40 µm) and high protein concentrations (<10 µm) (Schutyser and Van der Goot, 2011).

According to Whitaker and Tannenbaum (1977) and Owusu-Ansah and McCurdy (1991), starch granules are uniform in size and average 20 μm, while protein body sizes are 1 – 5 μm in legume seeds. Therefore, uniform size starch particles and small size proteins in legume seeds can provide better air separation compared to cereal grains which contain non-uniform starch granule sizes. Air classification is also more suitable for faba beans rather than field pea because faba bean has more uniform large starch granules (15 – 30 μm). On the other hand, field pea has small and medium size starch granules (0 – 20 μm). Therefore, field pea protein fraction can contain more starch (10.7%) than faba bean protein fraction (1.30%) (Gunawardena et al., 2010).

Coda (2015) was found differences of chemical composition in faba bean flour, faba bean protein fraction, and faba bean starch fraction in Table 4. The faba bean flour and its protein and starch fractions varied in chemical composition such as protein, starch, fiber, and ash content. Flour was characterized by 35.7 ± 0.4% of dry matter (DM) of protein and 42.1 ± 0.8% dm of starch. Protein fraction had the highest amount of protein (51.5 ± 0.2% dm) but also significantly higher content of dietary fiber, ash, and fat. Starch fraction had the highest amount of total starch (65.8 ± 0.5% DM) and the lowest fat concentration (Coda et al., 2015).

Table 4. Chemical composition of faba bean flour, faba bean protein fraction, and faba bean starch fraction. Results expressed as dry matter (DM) basis (Coda et al., 2015).

	Faba bean flour	Faba bean protein fraction	Faba bean starch fraction
Moisture %	9.45 ± 0.07[a]	7.66 ± 0.15[c]	8.53 ± 0.04[b]
Ash (% DM)	3.98 ± 0.04[b]	5.38 ± 0.01[a]	2.22 ± 0.02[c]
Protein (% DM)	35.66 ± 0.38[b]	51.49 ± 0.23[a]	16.73 ± 0.03[b]
Starch (% DM)	42.21 ± 0.77[b]	23.38 ± 0.18[c]	65.82 ± 0.54[a]
Fiber (% DM)	7.17 ± 0.32[b]	10.16 ± 0.23[a]	4.64 ± 0.14[b]
Fat (% DM)	1.53 ± 0.04[b]	2.00 ± 0.06[a]	0.85 ± 0.02[c]

The data are the means of three independent experiments ± standard deviations (n = 3).
a-e Values in the same row with different superscript letters differ significantly (P b 0.05).

2.5. Soybean meal

Globulins are the main storage proteins in soybean and globulin proteins are classified within four groups according to their sedimentation coefficients such as 2S, 7S, 11S and 15S (Nishinari et al., 2014). Still, the 7S and 11S proteins make up around 80% of storage proteins in soybean (Nishinari et al., 2014). In soybean seed, the 7S protein is known as β-conglycinin and the 11s protein is known as glycinin. β-conglycinin is a trimer of 150 – 200 kDA including three subunits such as α`, α, and, β and their molecular masses are 72 kDa of α`, 68 kDa of α, and 52 kDa of β (Lampart-Szczapa, 2001, Nishinari et al., 2014). The three subunits have similar AA sequences however, the content of cysteine, methionine, and tryptophan residues are different, and therefore, higher cysteine, methionine, and tryptophan containing subunits contain a higher nutritional value. The α` has a greater nutritional value than the α and β subunits and the α has greater nutritional value than the β subunit (Lampart-Szczapa, 2001). β-conglycinin is a glycoprotein and carbohydrates and one unit of aspartic acid residue are attached at the N-terminal end of the molecule (Lampart-Szczapa, 2001, Barać et al., 2004). Glycinin has a

hexamer shape and the molecular mass of glycinin is 300 – 380 kDa (Lampart-Szczapa, 2001). It contains five subunits (G1, G2, G3, G4, G5) and each subunit consist of an acidic subunit (A) with a molecular mass around 35 kDa and a basic subunit (B) of molecular mass around 20 kDa. Those acidic and basic subunits are connected to each other with disulfide bonds which consist of cysteine residue in each subunit sides (Lampart-Szczapa, 2001).

Storage of soybean is critical before the SBM process. Wright (1981) mentioned about McDonalds' soybean storage steps, soybean seeds should be dried at 79˚C until the moisture content of soybean seeds are around 13% and then soybean seeds should be dried at a lower temperature of 65˚C until moisture content of seeds becomes 9 – 10%. This process should take a minimum of 14 days. After this drying, the process of removing hulls becomes more efficient, but SBM can be produced without hull or with hull. Soybean seeds are grinded by the cracking rolls for reducing the beans' sizes of 1/6 – 1/8 soybean particles (Wright, 1981). The cracked beans pass through aspirators which separate hulls from cracked beans. The cracked beans are treated by heat at about 77˚C and moisture about 10% in the condition (Wright, 1981). Next, treated beans are flaked by flaking rollers. The purpose of flaking increases the surface area for extraction. After the flaking process, flaked beans are extracted with hexane to remove the soybean oil. After removing soybean oil, hexane residues in flaked beans should be desolventized. It can be desolventized and then cooked or both desolventized and cooked some time (Wright, 1981). The last step to obtain SBM is heat treatment such as an extrusion. Extrusion is the most common way to apply heat (120˚C) during SBM production. During SBM production, heat and moisture treatments are applied many times, therefore, reducing trypsin inhibitors, lectins, phytic acid, condense tannins, urease activity, and increased protein denaturation occurs (Wright, 1981, Alonso et al., 2000).

Soybean is a rich plant protein source for livestock and aquaculture animals. In spite of this, ANFs limit the usage of raw soybean in animal diets. There must be applied heat and moisture

treatment on soybeans before use in an animal diet. Raw soybean contains about 37% CP, 18% crude fat, and 6% crude fiber (Council, 2012). The carbohydrates constitute approximately 35% of the soybeans. Around 50% of carbohydrates are found in non-structural form such as low molecular weight sugars, oligosaccharides, and small amounts of starch, while the other 50% are structural polysaccharides, including a large amount of pectic polysaccharides (Karr-Lilienthal et al., 2005). The RFOs are about 5% of soybean and starch is less than 1% of soybean. The main animal feed product of soybean is soybean meal. There are two types of SBM founding on the market such as without hull and with hull. Dehulled SBM contains approximately 49% CP and 4% crude fiber (Council, 2012). On the other hand, the soybean with hull contains approximately 44% CP and 8% crude fiber (Council, 2012). Both dehulled and with hull soybean meals contain approximately 3% crude fat and 40% carbohydrates (Karr-Lilienthal et al., 2005). SBM contains well balanced AA composition in Table 5 and all essential AAs are found in SBM. As all *Fabaceae* family crops, SBM is rich in lysine and poor in methionine (Woyengo and Nyachoti, 2012). Lysine and methionine are the first two limiting essential AAs in farm animal diet.

Table 5. The amino acid composition of soybean meal (Woyengo and Nyachoti, 2012).

Indispensable amino acids of SBM (% DM basis)								
Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
3.31	1.24	2.02	3.78	3.30	0.66	2.39	1.92	2.13
Dispensable amino acids of SBM (% DM basis)								
Alanine	Aspartic acid	Cystine	Glycine	Glutamic acid	Proline	Serine	Tyrosine	
2.13	5.49	0.64	1.97	9.01	2.46	2.57	1.59	

2.6. Pellet press and extrusion

Pelleting is one of the most common processing technologies to give a specific shape for feed ingredients. In the late 1800s, the first feed service was needed for working horses and mules and later calf meal was demanded by US dairy farmers (Schoeff et al., 1994). Today, pelleted feed has been produced for poultry, ruminants, swine, equine, and aquatic animals. A

pelleted feed production line may consist of weight batching, grinding, mixing, conditioning, pelleting, and cooling instruments. The steam press conditioner is cylindrically shaped with different sizes, lengths, and layers. Paddles are attached to a rotating shaft to move the mash through the die and there are also inlets for steam and water to treat the mash. According to Svihus and Zimonja (2011), in conditioning, the grinded and mixed feed ingredients (mash) are treated by saturated steam while they are mixed and moved by paddles. During this process, the mash reaches around 75º C and the moisture content of mash increases by 3–4% units. The pellet press is located right after the conditioner. A pellet press in feed industry consists of rollers and a ring-shaped or flat die. After heat and moisture treatments in conditioner, the mash enters the pellet press. The rollers force the mash through cylindrical holes in a die and the mash is formed into pellet shape. The constant movement of rollers creates friction and due to friction, the temperature increases by up to 5-10˚C (Svihus et al., 2004). Some chemical change may occur in proteins during the pelleting process such as denaturation of proteins. Denaturation is the unfolding of proteins from their tertiary or secondary structures (Svihus and Zimonja, 2011). During pelleting, the destruction of a three-dimensional structure of proteins may occur due to the heat treatment. The denaturation of proteins can be described in two stages: reversible and irreversible. The breakage of hydrogen and van der Waal bonds is reversible but breakage or formation of covalent bonds such as the disulphide bridges is irreversible (Weijers and Van't Riet, 1992). Denaturation temperature may be different for different proteins. According to Adams (1991), at excess water content, most proteins start to denature at a temperature of 60 - 70˚C, however, some proteins may denature at temperatures as low as 40˚C while other proteins may remain inactive at 80˚C or higher. Enzymes and enzyme inhibitors are active when found in a tri-dimensional structure and covalent and non-covalent bonds provide their structure for catalytic or enzyme-binding activity. Temperature may destroy their bonds and thus their tri-dimensional structure and activities (Svihus and Zimonja, 2011). It is hard to conclude the effect of pelleting on protein digestibility because heat treatment eliminates the activity of protease enzyme

inhibitors and denatures proteins. Therefore, determining which of those have a direct effect on improving protein digestibility remains uncertain (Svihus and Zimonja, 2011). The physical pellet quality is an important factor for delivering a balanced diet. During the denaturation of proteins, gelling property of proteins may improve which may increase pellet durability and pellet quality (Svihus and Zimonja, 2011).

Extruder has been used for more than 70 years. The first screw extruder was used for continuous cooking in the late 1930s and the first commercial extruder was used for cereal grains in the mid-1940s in the United States (Rokey et al., 2010). Extrusion now becomes the major process to produce snack food, breakfast cereals, pasta, textured vegetable proteins, aquaculture feed, and pet food. Extruders can be produced in a variety of types, sizes, and modifications according to the industry which it is used. In the feed industry, a metal barrel has one or two screws which convey the materials in the barrel. There is a die end of the barrel which forms the final shape of the feed and two or more blades cut the product. In animal feed industry, extrusion process is found as a line system including a grinder, mixer, preconditioner, extruder, dryer, cooler, and coater. Denaturation of native proteins occurs during the extrusion process due to temperature and shearing effect. However, protein structure and process parameters also impact protein denaturation. In the extrusion process, the AA sequence of proteins determines the denaturation temperature with process parameters such as the feed material's composition, screw speed, barrel temperature profile, feed rates, and die size and shape, specific mechanical energy input, torque, pressure at the die, residence time, and the degree of screw fill (Verbeek and van den Berg, 2010). The denaturation of proteins concludes with unfolded protein molecules which are more suitable to digest by proteolytic enzymes such as trypsin and chymotrypsin and extrusion also inactivates protease enzyme inhibitors (Camire, 1991). Both denaturation of proteins and inactivated protease enzyme inhibitors can affect the protein digestibility. One of the main advantage of the extrusion process is reducing or removing ANFs (Table 6) such as

protease inhibitors, lectins, tannins, and phytic acid (Alonso et al., 2000) which can inhibit protein digestibility.

Table 6. Anti-nutritional factors in raw, dehulled, and extruded faba beans (Alonso et al., 2000).

Anti-nutritional factors	Faba bean (Vicia faba)		
	Raw seeds	Dehulling	Extrusion
In vitro protein digestibility (%)	70.8	72.5	87.4
Trypsin inhibitors (IU/mg DM)	4.47	4.99	0.05
Chymotrypsin inhibitor (IU/mg DM)	3.56	3.71	1.68
Lectins activity (IU/mg DM)	49.3	49.3	0.2
Phytic acid (g/kg DM)	21.7	23.8	15.9
Condensed tannins (g eq cat kt DM)	1.95	0.15	0.89

2.7. Broiler chicken digestive system

Chickens consume their feed by using their beak and send their feed to the mouth. There is no chewing motion in mouth due to lack of teeth in chickens. In the mouth, saliva glands provide moisture for helping swallowed the feed. Chickens use their tongues to push feed to the esophagus. The esophagus connects the crop to the mouth and proventriculus (Figure 6). It has a flexible tube therefore it can carry the feed efficiently.

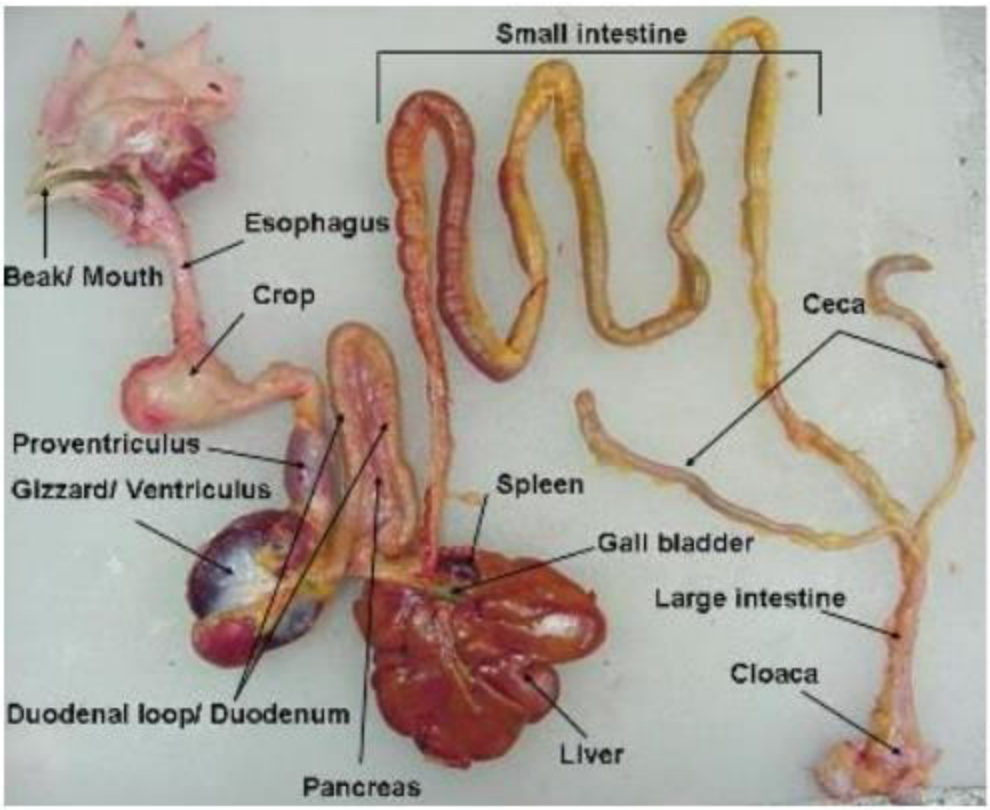


Figure 6. Digestive tract of a chicken (Jacob et al., 2011).

The crop is located in the neck region and it is shaped as a pocket. The feed and water can be stored in the crop or they can be passed the crop and send directly to the proventriculus. The capacity of crop is between 5-10 g (Svihus, 2014). The true digestion starts in the proventriculus. Hydrochloric acid (HCl), digestive enzymes (pepsinogen), and mucus are secreted by proventriculus and mix with the feed (Svihus, 2014). Pepsinogen transforms to the pepsin and HCl then, the digestion of proteins is started. The pH was measured to 2 in proventriculus (Duke, 1986). Ventriculus or gizzard are located next to the proventriculus. The main functions of gizzard are grinding, mixing, and mashing. The strong myolinated muscles and sand-paper like surface of the gizzard performs the grinding function (Svihus, 2014). The small intestine consists of the duodenum, jejunum, and ileum. The duodenum secretes bicarbonate and digestive enzymes (trypsin, chymotrypsin, carboxypeptidases A and B, proelastase, α-amylase, lipase, lecithinases,

and nucleases) from the pancreases and bile from the liver via the gallbladder (McDonald et al., 2011). When acidic contents from the proventriculus and gizzards are mixed with bile and bicarbonate, the pH in intestine increases to above 6 (Svihus, 2014). The bile activates pancreatic lipase and has a role in emulsifying the lipids. Starch digestion begins with pancreatic α-amylase in small intestine. The α-amylase enzyme attacks the α-(1→4)-glucan links in starch. Protease enzymes such as trypsin and chymotrypsin secrete from pancreases to hydrolase proteins. The pancreatic enzymes require a specific pH (pH 7-9) to active (McDonald et al., 2011). During protein digestion, each protease enzymes have a specific function. Trypsin enzyme works on peptide linkages involving the carboxyl groups of lysine and arginine (McDonald et al., 2011). Chymotrypsin enzyme acts upon peptide bonds involving the carboxyl groups of tyrosine, trptophan, phenylalanine, and leucine (McDonald et al., 2011). Carboxypeptidases enzymes attract the end of the peptide chain therefore they split of the terminal AA and turn α-carboxyl group free (McDonald et al., 2011). The jejunum and ileum are site for protein, starch, lipid, vitamin, and mineral digestion. The villi are finger-like component and its main function is absorption of the nutrients. The villi are able to produce some enzymes such as sucrose and maltase (McDonald et al., 2011). The pair of ceca is located where the ileum and large intestines join. There are two important functions of the ceca. First, the ceca absorbs the water remaining in the digested materials. Second, fermentation of remaining nutrients occurs in the ceca and as fermentation products, several volatile fatty acids and vitamins B such as (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and vitamin B12 are produced by microorganism (Jacob et al., 2011). However, few of the produced nutrients can be absorbed and is available for the chicken (Jacob et al., 2011). The large intestine is the last compart of the digestive system. The main function of the large intestine is to reabsorb water (McDonald et al., 2011).

3. MATERIALS AND METHODS

3.1. Diets preparation

Diets were manufactured by the Center for Feed Technology (FôrTek), at the Norwegian University of Life Science (NMBU), in Ås, Norway. Two different processes (pellet press and extrusion) were used to produce four diets in Table 7. Soybean meal diets were the control diet and were produced by pellet press (SBM-P) and extruder (SBM-E). SBM, wheat, and rapeseed oil were the main raw ingredients in control diets (SBM-P and SBM-E). Vertigo faba bean is used in this experiment. The air-classified faba bean protein (FBP) diets were the experimental diet and were also produced by pellet press (FBP-P) and extruder (FBP-E). In experiment diets (FBP-P and FBP-E), faba bean protein, wheat, cellulose, and rapeseed oil were used as raw ingredients. Titanium dioxide was also used as a digestibility marker.

Table 7. The composition of soybean meal (SBM) and faba bean protein (FBP) diets.

Raw ingridients, g/kg (as-fed)	SBM based diet	FBP based diet
Soybean meal	274	-
Faba bean protein fraction	-	193.93
Wheat	582	589
Rapeseed oil	75	76
Cellulose powder[1]	-	58
Limestone	14.76	16.50
Monocalcium phosphate	16.79	19
Sodium chloride	4.76	3.9
L-Lysine	8	14
DL-Methionine	6.10	7.50
L-Threonine	4	7.58
Choline chloride	1.96	1.96
Premix (Mineral &Vitamin)[2]	6.13	6.13
TiO2	5	5
Enzyme[3]	1.5	1.5

[1] SANACEL® 150, CFF GmbH & Co. KG, Gehren. Germany.

[2] Mineral and vitamin premix provided the following per kg diet: Fe, 50 mg; Mn, 122 mg; Zn, 80 mg; Cu, 14 mg; I, 0·72 mg; Se, 0·28 mg, retinyl acetate, 5.72 mg; cholecalciferol, 0.15 mg; dl-α-tocopheryl acetate, 78 mg; menadione, 8 mg; thiamine, 5 mg; riboflavin, 24 mg; niacin, 32 mg; calcium pantothenate, 24 mg; pyridoxine, 13 mg; cobalamin, 0.03 mg; biotin, 0.5 mg; folic acid, 4 mg.

[3] Enzyme Rovabio Excel Ap T-Flex, Adisseo, France provided the following per kg diet: Endo-1,4-β-xylanase: 33 000 visco units; Endo-1,3(4)-β-glucanase: 45 000 visco units; Endo-1,4-β glucanase (cellulase) >9600 DNS units + 16 other enzyme activities obtained from a fermentation broth of Penicillium funiculosum.

3.2. Feed production

3.2.1. Dehulling, air classification, grinding, and mixing

Whole faba beans were dehulling by a multistep process starting with grinding whole beans trough a roller mill (DT900-12; CPM-Roskamp, Waterloo, IA, the United States) with an 8 mm gap between rolls. The cracked faba beans were then passed through a Type Vibam 1013 (Damas A/S, Faaborg, Denmark) to remove dust, then smaller particles were removed by a Triør Type Hotyp 520 (Damas A/S, Faaborg, Denmark), and uncracked faba beans and split beans with hulls were removed by a vibration table (Sorla SB, Damas A/S, Faaborg, Denmark). The dehulled beans were milled with a Contraplex 630 C pin mill (Hosokawa Alpine, Augsburg, Germany) and air-classified into a light (protein) fraction and a heavy (starch) fraction by using an Air Classifier 500 ATP (Hosokawa Alpine, Augsburg, Germany. Wheat was also milled with a Contraplex 630 C pin mill (Hosokawa Alpine, Augsburg, Germany) and SBM was grinded with a Münch Hammermill (HM 21.115, Wuppertal, Germany) by using a 1 mm screen at FôrTek. All macro ingredients which required grinding were milled by using a hammer mill. Cellulose and macro minerals such as limestone were bypassing the grinding process. Macro ingredients were sent to a 400 liter (l) mixer (Twin shaft paddle, Tatham of England, 7.5 kW) and were mixed 180 seconds (3 min). A 20 l stainless steel pressure tank was used at 4 bars to spray the rapeseed oil by a nozzle (angle 65, size 05, Unijet, spraying systems Co, Wheaton, Illinois, USA) and spraying time was at 7.6 min. The mixing time post rapeseed oil addition was 120 seconds (2 min) with micro ingredients. Micro ingredients were prepared manually by mixing vitamins, AAs, micro minerals, premix, enzyme and marker. The premix mixtures were added manually during a third mixing cycle into the mixer. The total duration time of mixing for each batch was 12.6 min.

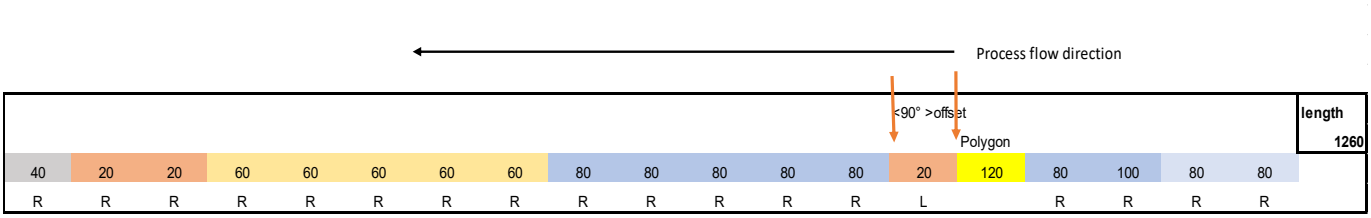
3.2.2. Pellet press and extruder

To produce pelleted broiler feed, the mixed mash was sent through the double conditioner (Twin Pass, Muench, Germany, 1.2 t/h, 2 x 1.8m x 30cm). The steam pressure was set to 8 bars and working pressure at pellet press was set to 2.3 bars. 4% steam was added to the mash. After being pre-treated in the conditioner, the mash was processed in a pellet press (Muench, Germany, 1.2 t/h max. capacity, 2 x 18.5 kW). The roller and die distance in the pellet press was 0.5 mm and the die's sieve was 3x42 mm at a production rate of 400 and 200 kg/h for the wheat-based and the faba bean protein fraction diet respectively. Immediately after the pelleting, the temperatures of feed were measured manually with a thermometer in an insulating box. The pelleted feed was sent to the cooler (Miltenz, New Zealand, capacity 1.2t/h) directly. A counter-flow cooling system was used for 45 min, which used ambient air to reduce temperature of the products.

Before extrusion process started, the mixed mash feed was carried through an extruder bin. The mixed mash was sent to a Bühler pre-conditioner (BTCT) (Bühler, Uzwil, Switzerland) where water and steam were added to the mash. Retention time for pre-conditioning was 62 seconds. All diets were extruded in a twin-screw co-rotating 5 barrel extruder (Bühler BCTG 62) driven by a 45 kW electrical motor. Maximum production capacities of the extruder were 287 kg/h for SBM based diet and 211 kg/h for faba bean protein fraction based diet of broiler feed. Capacity was regulated by feeder screw speed at 145 kg/h. Die plate contained 12 dies in 3 mm size. In barrel 5, cold water was used to decrease temperature of the mash.

The experiment was designed with only one screw configuration for all diets. The screw configuration was shown in Figure 7. After extrusion, a dryer (FôrTek design fluid bed dryer) was used in order to reduce moisture of the extruded pellets. The dryer was used dry air which 110˚C in the 1st segment and 65 ˚C in the 2nd segment of dryer and the belt speed of dryer was set 3% which was 10 min. The obtained extruded pellets were air-dried in a bed dryer fixed with electrical

fans to achieve final dry matter (96-97% DM). Additional drying for extruded pellets were performed manually by using 40 kg batch dryers/coolers made by “FôrTek”. The fan was a W2E 300-DA 01 W-160(EBM-Papst, Mulfingen, Germany) with capacity 2550 m³/h. The heater was “Viking” (Viking, Denmark), power 10 kW with max air temperature 60°C. Drying was performed at a temperature of 60°C, for 45 min for each 40 kg batch. Cooling was performed in the same dryers/coolers by switching off the heater, at room temperature of 20°C, 5 min for each batch.



R - Right turn of the element

L - Left (backward) turn of the element

UC - **U**nder **C**ut (means more free volume in the extruder)

Numbers are representing the lenght of the Archimedean Spiral (screw alike) for each screw element

Red arrow means a spacer

Total length of the screw is 1260 cm

Figure 7. Screw configuration for extrusion process

3.2.3. Feed sampling

The mixed mash samples were taken before heat treatments (pellet press or extrusion). Four samples from each diet were taken after the mixing process. The mixed samples were taken directly from the waiting hopper which is under the mixer. The samples were taken from different spots and mixed together in a bucket to achieve representative samples and finally transferred into plastic bags for further investigations.

The representative pellet samples from each diet were taken directly from the filled bags with a grain sampler (A/S rationel cornservice, Esbjerg/Denmark). The extruded pellets were taken after the drying process. The samples were taken from different spots and mixed together in a bucket to achieve representative samples, finally transferred into plastic bags. About 1 kg

pellets of each diet were collected for chemical and physical analysis. The samples were kept at 4ºC for further assessments.

3.3. Broiler chicken management and sample collection

3.3.1. Broiler chicken management

Broiler chicken feeding trial was carried out in strict accordance with the recommendations of the National Ethic Commission (Warsaw, Poland). All experimental procedures complied with the guidelines and were approved by the Local Ethic Commission of the Poznan University of Life Sciences (Poznan, Poland) with respect to animal experimentation and care of animals under study. Broiler chicken trial started on 22.01.2018 and ended on 22.02.2018.

A total of 400 one-day-old male broilers (Ross 308) were randomly allocated to 40 floor pens (1 x 1 m) that were bedded with chopped wheat straw (7-15 cm length) and contained 10 birds each. The pens were arranged in the center of an environmentally-controlled broiler house (PIAST PASZE Sp. z o.o., Experimental Unit Olszowa, Poland) that contained 9000 birds of the same age as those in the experiment. A temperature of 33˚C was maintained during the first week, then reduced by 3-4˚C weekly to a minimum temperature of 21˚C. The birds were maintained on a commercial pelleted diet produced by the Piast Pasze factory (Lewkowiec, Poland) until 16 day, and fresh water was provided ad libitum throughout the experimental period. At 17 day, the 400 birds were randomly distributed among 4 dietary treatments using 10 replicate pens per treatment and 5 birds per pen.

3.3.2. Sample collections from broiler chicken

At the age of 30 days, 20 birds (2 birds/replicate pen) per treatment were weighed, killed by cervical dislocation. Next, using clamping forceps, the jejunum and ileum were clamped at

three points (start, middle, end) to prevent the passage of contents along the intestine, then weighed. The jejunum was defined as the segment from the end of the duodenal loop to Meckel's diverticulum, and the ileum as the section from Meckel's diverticulum to the ileocecal junction. Each of the two segments was then divided into two parts of equal length: upper and lower jejunum (UJ and LJ), upper and lower ileum (UI and LI) and the contents of each part were expressed by gentle manipulation into a pre-weighed plastic container and stored at −20°C until analyses.

3.4. Physical and chemical analyses

Physical analysis of pelleted and extruded broiler feeds were measured including pellet durability index (PDI), particle size distribution, hardness, and water stability at Feed lab, NMBU.

3.4.1. Pellet durability index

Durability was measured using Holmen pellet tester (NHP200). Surface attrition was measured as the pellets were conveyed at high air velocity with reference to time. Attrition of surface occurs when pellets hit pipe walls, bends, and other pellets. 100 g pellets were taken and the Holmen was run with a die size setting as 3 mm for pelleted diets, and 4 mm for extruded diets. The dust was collected automatically by the machine via sieving. After test, the final weight of the diets was recorded. Pellet durability index (percentage) was calculated using the following formula. Two replications were taken for measurement.

PDI (%) = final weight of pellets after Holmen (g)/weight of pellets before Holmen (g) x (100)

3.4.2. Hardness

Hardness was measured on a Lloyd texture analyzer (Model 1000R, Hampshire, UK) equipped with a 50N load cell using a compression speed. The hardness value was given in force

(N) at breakage point. Diameter and length of 30 pellets were recorded to take the average for each treatment, and then 15 pellets from each diet were chosen to test hardness according to these averages. Expansion was calculated using the following formula. 15 pellets from each diet were chosen for measuring expansion.

((pellet width–die diameter) / die diameter) x 100.

3.4.3. Water stability test

Water stability test was done the same methods as described by (Baeverfjord et al., 2006). 50 g samples of pellets of each diet were sieved and 10 g sieved sample were placed in a wire netting basket, and slowly immersed in a glass beaker containing 300 ml tap water. Each wire net basket was weighted before testing to calculate the final weight. Analyses were done at 24.5°C with 120 rpm for 30 min. The sieves were removed from the beaker, and wire baskets containing crumbles allowed to drain for 1 min, oven-dried at 105°C for overnight, cooled in a desiccator and reweighed. Water stability was calculated as the percentage difference in sample weight inside the wire baskets after reweighing and expressed as % loss of dry matter.

3.4.4. Particle size distribution

Particle size analyses were performed for mixed mash by using a Malvern Mastersizer 2000 laser diffraction analyzer (Malvern Instruments Ltd, Malvern, United Kingdom) which can measure particles > 0.02 µm - 2000 µm. The results were expressed by d (0.1), d (0.5) and d (0.9). The mixed mash samples from the waiting hopper under mixer were used for each diet. The particle size distribution analysis was done for two duplicates for each diet. System adjustments and corrections were conducted before each measurement. Water was used as the dispersant.

3.4.5. Chemical analyses

The representative feed samples (n=3) were ground on a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55743 Idar-Oberstein, Germany) through a 0.5 mm sieve and the digesta samples of chicken were grinded gently by hand in Poznan, Poland. They were analyzed by the LabTek group for dry matter, ash, crude protein (Kjeldahl-N *6.25), starch, NDF, crude fat, starch, gross energy, titan, amino acids, protein digestibility, and enzyme analysis at the Department of Animal an Aquacultural Science (IHA), NMBU, Ås, Norway.

Dry matter (DM) of the feeds was determined after drying loss to constant weight in an oven at 103 °C for overnight (ISO 6496: 1999). Crude ash content was analyzed by combustion until constant weight at 550 °C for maximum 20 hours (ISO 5984: 2002). CP (Kjeldahl-N *6.25) which was determined using a 2400/2460 KjeltecTM Auto Sampler and the Kjeltec 1015 Digester Tecator (FOSS Analytical, Hilleroed, Denmark), according to Commission regulation (EC) No 152/2009. Starch content was analyzed using an enzymatic-colorimetric method by McCleary et al. (1994). In summary, starch and starch granules were degraded with α-amylase and amyloglucosidase enzymes to glucose. Then, glucose concentration was determined using a spectrophotometer (MaxMat PL II Multianalyzer, France). Neutral detergent fiber (NDF) content was analyzed using the Ankom200 Fiber Analyzer (ANKOM Technology, Macedon, New York, USA) according to Mertens (2002). Crude fat was analyzed after extraction with 80% petroleum ether and 20% acetone in an Accelerated Solvent Extractor from Dionex (ASE200; Sunnyvale, CA, USA). Gross energy (GE) content was determined by using a PARR 1281 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL, USA) (ISO 9831, 1998). AAs in pelleted and extruded feeds were analyzed using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK) by Commission regulation (EC) No 152/2009. NSP content of faba bean protein (air-classified) was analyzed by the Englyst NSP procedure with HPLC/GC analysis. Freeze-dried jejunal and ileal digesta contents were pulverized using a mortar and pestle, and the contents from two birds

per replicate-pen were pooled and analyzed in duplicates for nitrogen and TiO2. Nitrogen contents were analyzed by the Dumas method using a Vario El Cube (Elementar Analysensysteme GmbH, Hanau, Germany 2016) and titanium dioxide content was determined as described by Short et al. (1996). The digesta content samples from the lower jejunum are taken from one bird from each pen and prepared as described by Pérez de Nanclares et al. (2017) for enzyme activities analysis. Trypsin activities were assayed colorimetrically using trypsin commercial assay kits (Abcam, Cambridge, UK) according to the manufacturer's instructions. Activities of trypsin were expressed as unit/g jejunal chyme.

3.5. Calculation and statistical analysis

Statistical analyses were accomplished using the Minitab 18. A two-way ANOVA analysis of variance was performed to determine the main effects and interactions of protein sources and processing methods (as independent variables) on growth parameters, protein digestibility and trypsin enzyme activity. Means were separated by Tukey and differences were considered significant at $P < 0.05$. Pen means (5 birds) were used as the experimental unit for performance data. The equation was used to calculate the protein digestibility (D) of the control and experimental diets:

$$\text{Dprotein} = \left\{1 - \left[\left(\frac{\text{TiO}_2\text{(g/kgdiet)}}{\text{TiO}_2\text{(g/kgdigesta)}} \right) \text{x} \left(\frac{\text{protein (g /kgdigesta)}}{\text{protein (g/kgdiet)}} \right) \right] \right\}$$

4. RESULT

4.1. Diets

The analyzed composition of raw faba bean and its air-classified fractions such as dehulled bean, hull, protein and starch fractions are shown in Table 8. Faba bean protein fraction contained more crude protein, fat, and ash than the other analyzed values. Faba bean hull had the highest NDF and the starch fraction contained more starch than other analyzed samples.

Table 8. Chemical composition of whole faba bean (whole FB), dehulled faba bean (dehulled FB), hull, and protein and starch fraction after air classification of dehulled beans.

Parts and fractions of faba bean seeds	Dry Matter	Crude protein	Starch	NDF	Crude Fat	Ash
	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
Whole FB	856.47	293.06	376.74	176.20	19.98	44.43
Dehulled FB	860.16	321.02	359.71	56.45	20.28	49.75
Hull	860.25	63.64	-	619.37	2.69	26.30
Protein fraction	924.53	632.41	87.88	98.13	32.99	65.78
Starch fraction	902.15	176.66	744.42	21.69	7.94	21.35

NSP content of faba bean fractions and neutral sugars of faba bean protein fraction are shown in Table 9. Faba bean hull contained more total, insoluble, soluble NSP, and NDF than protein and starch fractions. Faba bean protein fraction contained more: 11% of total, 6.6% of insoluble, and 4.3% of soluble NSP compared with faba bean starch fraction: 4.2% of total, 2.2% of insoluble, and 2% of soluble NSP. Neutral sugars of faba bean protein fraction was analyzed as total, insoluble, and soluble. Arabinose contained the highest total NSP with 55.06 g/kg DM and followed by uronic acid (17.84 g/kg DM), glucose (17.66 g/kg DM), galactose (8.64 g/kg DM), xylose (7.05 g/kg DM), rhamnose (2.61 g/kg DM), and fucose (0.74 g/kg DM). Neutral insoluble sugars were listed from the highest to the lowest by arabinose (33.77 g/kg DM), glucose (15.58 g/kg DM), uronic acid (6.26 g/kg DM), xylose (5.45 g/kg DM), galactose (3.35 g/kg DM) rhamnose (1.27 g/kg DM), fucose (0.60 g/kg DM). Neutral soluble sugars were listed from the highest to the

lowest by arabinose (21.32 g/kg DM), uronic acid (11.57 g/kg DM), galactose (5.29 g/kg DM), glucose (2.08 g/kg DM), xylose (1.61 g/kg DM), rhamnose (1.34 g/kg DM), fucose (0.14 g/kg DM). Mannose was not detected.

Table 9. NSP percent of faba bean hull, protein, and stach fraction and neutral sugar content of faba bean protein fraction.

Faba bean fractions	Total NSP (% of DM)	Unsoluble NSP (% of DM)	Soluble NSP (% of DM)	NDF (% of DM)
Faba bean hull	56.0	50.0	6.0	53.3
Faba bean protein fraction	11.0	6.6	4.3	9.1
Faba bean starch fraction	4.2	2.2	2.0	2.0

Neutral sugar	Faba bean protein fraction		
	Total NSP (g/kg DM)	Unsoluble NSP (g/kg DM)	Soluble NSP (g/kg DM)
Uronic acid	17.84	6.26	11.57
Rhamnose	2.61	1.27	1.34
Fucose	0.74	0.60	0.14
Arabinose	55.09	33.77	21.32
Xylose	7.05	5.45	1.61
Mannose	0.00	0.00	0.00
Galactose	8.64	3.35	5.29
Glucose	17.66	15.58	2.08

The analyzed chemical compositions of pelleted and extruded feeds are presented in table 10. Chemical analyses of diets comprise small variations in chemical composition results. SBM diets contain higher CP than FB diets (251.6 and 229.3 g/kg DM vs. 219.2 and 216.4 g/kg DM). FBP-E has the highest fat content (117.2 g/kg DM) compared to the other diets. NDF has the largest variation between chemical analyses. FBP diets contain higher NDF 134.0 and 130 g/kg DM respectively compared with SBM diets 107.8 and 106.7 g/kg DM. AA content is similar between pelleted and extruded diets.

Table 10. Analyzed chemical content of soybean meal pelleted (SBM-P), faba bean protein pelleted (FBP-P), soybean meal extruded (SBM-E), and faba bean protein extruded (FBP-E) diets.

	Diets			
Analysis	SBM-P	FBP-P	SBM-E	FBP-E
Dry matter	904.3	916.3	972.5	962.5
Crude Protein (g/kg DM)	251.6	219.2	229.3	216.4
Starch (g/kg DM)	375.8	399.8	385.6	393.0
Fat (g/kg DM)	88.4	99.9	88.5	117.2
NDF (g/kg DM)	107.8	134.0	106.7	130.0
Gross energy (MJ/kg DM)	19.7	19.7	19.8	20.2
Indispensable				
Lysine (g/kg DM)	18.0	20.8	19.2	19.4
Methionine (g/kg DM)	8.3	9.3	9.5	8.4
Threonine (g/kg DM)	11.5	14.4	12.5	13.4
Arginine (g/kg DM)	14.0	14.5	13.8	13.6
Leucine (g/kg DM)	16.0	14.5	15.7	13.2
Isoleucine (g/kg DM)	9.5	8.1	9.1	7.3
Phenylalanine (g/kg DM)	10.3	8.8	10.3	8.2
Histidine (g/kg DM)	5.2	4.8	5.1	4.4
Valine (g/kg DM)	10.6	9.6	10.3	8.8
Dispensable				
Cystine (g/kg DM)	3.0	2.7	3.1	2.5
Alanine (g/kg DM)	8.1	7.2	7.8	6.6
Serine (g/kg DM)	11.3	10.3	11.2	9.4
Tyrosine (g/kg DM)	5.4	4.6	5.8	4.9
Proline (g/kg DM)	14.3	13.5	14.8	12.5
Glycine (g/kg DM)	8.9	8.1	8.7	7.4
Aspartic acid (g/kg DM)	21.8	18.0	20.5	16.2
Glutamic acid (g/kg DM)	46.3	43.4	47.9	40.0

4.2. Feed processes

In the pelleting process (table 11), when the feeder speed was adjusted at 18 and 7%, the capacity of SBM and FBP diets became 400 and 200 kg/h respectively. Therefore, the difference in capacity caused by the feeder speed. Conditioner temperature kept constant at 81˚C however, SBM diet required more steam (29.5 kg/h) than FBP diet (19.6 kg/h). The differences of capacity and particle sizes can determine slightly different pellet temperatures between SBM diet at 89.2˚C and FBP diet at 93˚C.

Table 11. Pellet press parameters of soybean meal (SBM) and faba bean protein (FBP) diets.

		Diets	
Parameter	Unit	SBM	FBP
Die specification	mm	3 x 42	3 x 42
Feeder speed	%	18	7
Capacity	kg/h	400	200
Conditioner temperature	ºC	81	81
Pellet temperature	ºC	89.2	93
Motor load	%	16	14
Conditioner steam	kg/h	29.5	19.6
Ampere motor 1	amp	13	12.5
Ampere motor 2	amp	12	11
Total ampere motor	amp	118.7	112.6
Energy consumption, P. press	kW	73	69.3
Specific energy consumption, P. press	kWh/t	182.5	346.3

In the extrusion process (Table 12), conditioner temperature, feeder rate, specific mechanical energy (SME) and barrels' temperature held steady for both SBM and FBP diets. The differences between those parameters were small. Moisture amount and screw speed were varied to obtain better pellet quality. Screw speed and torque (%) had the largest differences between

SBM (475 rpm and 43%) and the FBP diet (599 rpm and 34.6%). SBM diet had lower level of steam and water in conditioner (8.5 and 13 kg/h) compared with the FBP diet (10.2 and 17.5 kg/h).

Table 12. Extrusion parameters of soybean meal (SBM) and faba bean protein (FBP) diets.

Parameter	Diets	
	SBM	FBP
Preconditioner		
Conditioner steam (kg/h)	8.5	10.2
Conditioner water (kg/h)	13	17.5
Conditioner temperature (°C)	89	90
Extruder		
Die size	3	3
Number of dies	12	12
Calibration (kg/h)	287	211
Feeder (kg/h)	145	145
Screw speed (rpm)	475	599
Torque (Nm)	192	151
Torque (%)	43	34.6
Drive power (kW)	9.4	9.7
SME (Wh/kg)	59.7	58.4
Barrel 1 (°C)	94.7	97.9
Barrel 2 (°C)	113.3	110
Barrel 3 (°C)	93	100.7
Barrel 4 (°C)	89.2	95.2
Barrel 5 (°C)[1]	65	67.4
Die temperature (°C)	91	90
Die pressure (bar)	30	24.5
Knife speed (rpm)	650	678
Number of knifes	6	6

[1] Barrel 5 contained cooling (3600 in 36 sec 1800)

4.3. Physical quality of feed

The analyzed physical pellet quality results for hardness, durability, water stability, expansion, length, and diameter are shown in Table 13. Pelleted diets had higher hardness than extruded diets. FBP-P had the highest hardness of 63.92 N and SBM-E had the lowest hardness of 39.45 N. The highest durability was 95.45% of FBP-E. Pellets from the SBM-P diet had higher durability than SBM-E (94.3 and 92.7% respectively). Pelleted feed had a higher water stability than extruded feed. FBP-P had the highest water stability with 86.45% and SBM-E had the weakest feed with 15.21% according to the water stability test. Pelleted diets did not expand. On the contrary, they shrinked. On the other hand, expansion was observed on extruded diets. SBM-E diet had 37.67% of expansion and FBP-E had 34.09% of expansion. Pelleted diets had longer length than extruded diets and diameter of pelleted diets were 2.91 mm compared with extruded diets 4.08 and 4.03 mm respectively.

Table 13. Physical quality of soybean meal pelleted (SBM-P), faba bean protein pelleted (FBP-P), soybean meal extruded (SBM-E), and faba bean protein extruded (FBP-E) diets.

Treatment	Hardness (N)	Durability (%)	Water stability (%)	Expansion (%)	Length (mm)	Diameter (mm)
SBM-P	63.92 ± 2.45	94.3	81.40 ± 0.548	(-3.98)±0.20	7.14 ± 0.139	2.91 ± 0.003
FBP-P	108.17 ± 4.52	93.7 ± 0.1	86.45 ± 1.418	(-3.62)±0.22	6.16 ± 0.191	2.91 ± 0.006
SBM-E	39.45 ± 0.97	92.7 ± 0.1	15.21 ± 0.737	37,67±0.65	5.45 ± 0.081	4.08 ± 0.021
FBP-E	44.99 ± 1.28	95.45 ± 0.05	34.13 ± 4.965	34.09±2.31	5.75 ± 0.166	4.03 ±0.029

Particle size distribution test by Master sizer of SBM diets and FBP diets result is shown in the Figure 8. SBM diets contained 5.9% of particles smaller than <10 μm, 11.6% of particles between 10-30 μm, 11.2% of particles between 30-60 μm, 12.2% of particles between 60-120 μm, 7.2% of particles between 120-210 μm, 11.7% of particles between 210-400 μm, 19.4% of particles between 400-720 μm, 16.1% of particles between 720-1260 μm, and 4.6% of particles between 1260-2200 μm. On the other hand, FBP diets contained 11.9% of particles smaller than <10 μm, 27.5% of particles between 10-30 μm, 20% of particles between 30-60 μm, 15.3% of

particles between 60-120 µm, 7.4% of particles between 120-210 µm, 7.4% of particles between 210-400 µm, 6.8% of particles between 400-720 µm, 3.4% of particles between 720-1260 µm, and 0.2% of particles between 1260-2200 µm. SBM diets had less percentage of particles between 1-210 µm with 48.1% compared with FBP diets 82.2%. However, SBM diets contained larger percentage of particles between 210-2200 µm with 51.9% compared with FBP diets 17.8%.

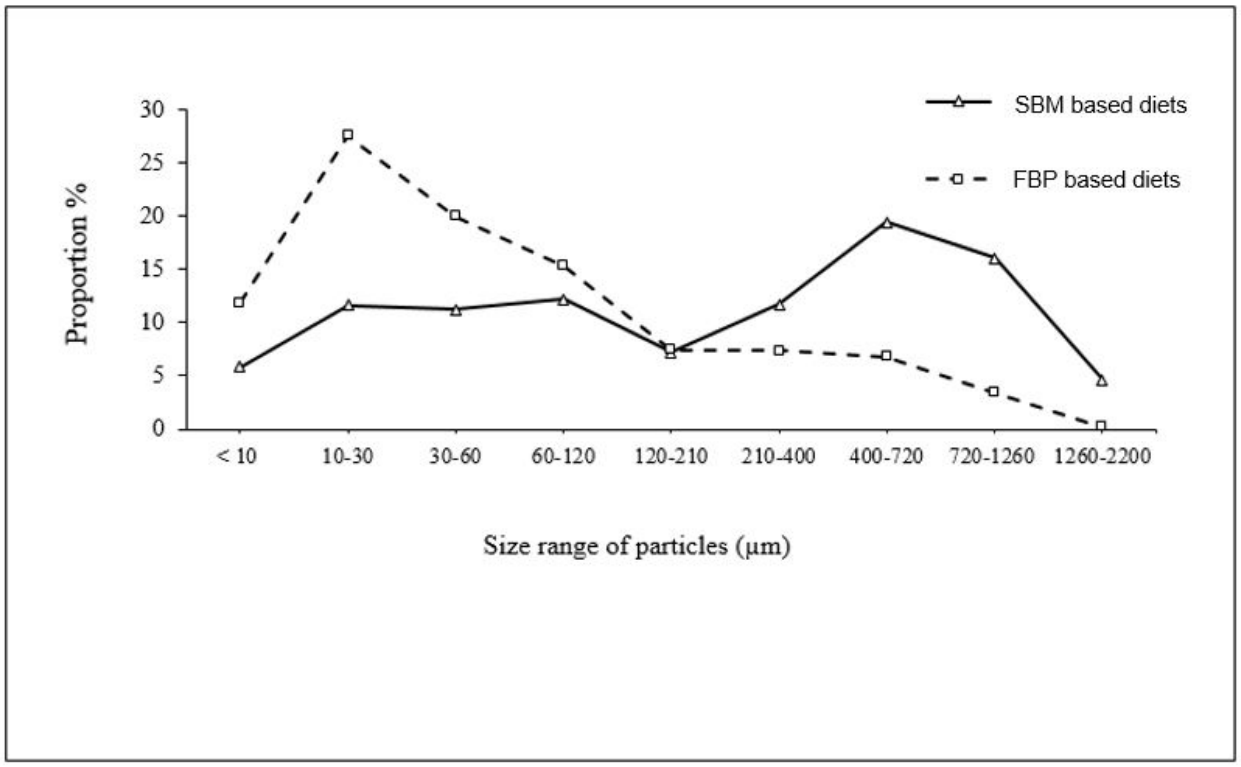


Figure 8. Particle size distribution of soybean meal (SBM) and faba bean protein (FBP) based diets.

4.4. Broiler chicken performance

The chickens stayed healthy and mortality was not observed during the 30 day's experiment. The effect of diets (SBM-P, FBP-P, SBM-E, and FBP-E) on broiler chicken as body weight (BW), feed intake (FI), and feed conversion ratio (FCR) is shown in Table 14. Pelleting

and extrusion process had no effect on body weight however, protein source in diet had a significant effect on body weight in all periods (17 – 23, 24 – 29, and 1 – 29). SBM-E had the highest body weight (2240.4 g) following SBM-P (2200.6 g), FBP-P (1724.3 g), and FBP-E (1704.5 g) during 1 – 29 day.

For faba bean diet, process had negative effect on the feed intake during 17 – 23 days. FBP-P had higher feed intake than FBP-E between days 17 – 23. On the other hand, process had no effect on feed intake, and feed intake was only affected by a protein source during 24 – 29 and 1 – 29 days.

During 17 – 23 days, there was no interaction between the process and protein source for FCR values. Extruded diet groups had lower FCR values during 17-23 days due to the lower feed intake for extruded feed groups. However, process had no effect on FCR, whereas protein source had an effect on FCR 1 – 29 days. The lowest FCR value was observed by SBM-E (1.233) and SBM-P (1.237), following FBP-E (1.353) groups. The highest FCR value was obtained by FBP-P (1.404) group.

Table 14. The growth performance of broiler chicken of fed by soybean meal pelleted (SBM-P), faba bean protein pelleted (FBP-P), soybean meal extruded (SBM-E), and faba bean protein extruded (FBP-E) diets.

Diet	Body weight (g)				Feed Intake (g)				Feed conversion ratio			
	1 - 16 days	17 - 23 days	24 - 29 days	1 - 29 days	1 - 16 days	17 - 23 days	24 - 29 days	1 - 29 days	1 - 16 days	17 - 23 days	24 - 29 days	1 - 29 days
SBM-P	686,7	671.8^{a}	842.1^{a}	2200.6^{a}	855,8	857.4^{a}	1002.1^{a}	2715.3^{a}	1,247	1.280^{b}	1.21^{b}	1.237^{b}
FBP-P	692,5	544.5^{b}	484.3^{b}	1724.3^{b}	865,2	749.6^{b}	797.0^{b}	2411.8^{b}	1,250	1.379^{a}	1.751^{a}	1.404^{a}
SMB-E	708,2	702.8^{a}	859.5^{a}	2270.4^{a}	855,3	847.0^{a}	1093.1^{a}	2795.4^{a}	1,210	1.209^{c}	1.285^{b}	1.233^{b}
FBP-E	708,0	553.6^{b}	443.0^{b}	1704,5^{b}	865,6	702.1^{c}	736.5^{b}	2304.2^{b}	1,223	1.275^{bc}	1.704^{a}	1.353^{a}
SEM	4,0	13,3	35,1	45,3	3,27	12,0	28,5	37,4	0,075	0,015	0,065	0,017
p - value	0,133	<0.05	<0.05	<0.05	0,532	<0.05	<0.05	<0.05	0,007	<0.05	<0.05	<0.05

4.5. Protein digestibility and trypsin enzyme activity

Protein digestibility of diets in the upper jejunum (UJ), lower jejunum (LJ), upper ileum (UI), and lower ileum (LI) are presented in Table 15. In UJ, the highest protein digestibility was observed by FBP-P and following SBM-P, FBP-E, and SBM-E. Protein source did not affect the protein digestibility but pelleting process had a positive effect on protein digestion in UJ. In LJ, process had no interaction with protein digestibility however protein source designated the protein digestibility. FBP diets had higher protein digestibility than SBM diets in LJ. In UI and LI, protein digestibility was affected by a protein source. There was no significant effect of the process on protein digestibility. FBP diets had the higher protein digestibility than SBM diets. The end of the small intestine (LI), FBP-E had highest protein digestibility followed by FBP-P, SBM-E, and SBM-P.

Table 15. Protein digestibility of soybean meal pelleted (SBM-P), faba bean protein pelleted (FBP-P), soybean meal extruded (SBM-E), and faba bean protein extruded (FBP-E) diets.

Diet	Processing	Jejunum		Ileum	
		Upper	Lower	Upper	Lower
SBM-P	Pelleting	0.370	0.582	0.711	0.813
FBP-P	Pelleting	0.447	0.708	0.826	0.875
SBM-E	Extrusion	0.254	0.538	0.737	0.824
FBP-E	Extrusion	0.265	0.688	0.825	0.902
√MSE*		0.110	0.061	0.039	0.030
Protein source					
SBM		0.312	0.560	0.724	0.818
FBP		0.356	0.698	0.825	0.888
Processing					
Pelleting		0.409	0.645	0.769	0.844
Extrusion		0.260	0.613	0.781	0.863
P-value					
Protein source		0.26	< 0.05	< 0.05	< 0.05
Processing		< 0.05	0.111	0.345	0.068
Protein source x processing		0.398	0.565	0.308	0.407

*√MSE: square root of means square error in the analysis of variance.

Trypsin enzyme activity of pelleted and extruded diets are shown in Table 16. FBP-E had the highest trypsin activity (5.33 U/g chyme) followed by SBM-E (4.74 U/g chime), FBP-P (4.20 U/g chime), and SBM-P (4.08 U/g chyme). Diet and process had no significant effect on trypsin activity. However, extruded diets contained higher trypsin enzyme activity in chyme compared with pelleted diets.

Table 16. Trypsin enzyme activity of soybean meal pelleted (SBM-P), faba bean protein pelleted (FBP-P), soybean meal extruded (SBM-E), and faba bean protein extruded (FBP-E) diets.

Diet	Processing	Trypsin (U/g chyme)	√MSE*
SBM-P	Pelleting	4.084	0.89
FBP-P	Pelleting	4.199	2.74
SBM-E	Extrusion	4.738	0.95
FBP-E	Extrusion	5.328	1.39
Diet			
SBM		4.411	
FBP		4.760	
Processing			
Pelleting		4.142	
Extrusion		5.033	
P-value			
Diet		0.522	
Processing		0.081	
Diet x processing		0.675	

Values are means of 10 replicate cages of 1 bird each
*√MSE: square root of means square error in the analysis of variance.

5. DISCUSSION

5.1. Diets

The chemical analyses showed that FBP is a good source of protein (632.41 g/kg DM). The CP content of FBP was higher than the study reported by Coda (2015) but CP content was lower than the study done by Gunawardena (2010). However, it may be misleading to use the CP content as the only measurement because different cultivations of faba bean have varied CP contents. Protein enrichment factor is used to measure the efficiency of air classification process and the CP content of air-classified fraction divided by the CP content of the ingredient (Bergthaller et al., 2001). The protein enrichment factor of FBP presented in this study was 2.2 and was equal to the amount (2.2) obtained by Gunawardena et al. (2010) but greater than the amount (1.4) obtained by Coda et al. (2015). Starch content was 87.88 g/kg DM and lower than (Coda et al., 2015) (233.8 g/kg DM) but higher than (Gunawardena et al., 2010) (1.39 g/kg DM). FBP contained twice the CP of faba bean seeds (Warsame et al., 2018) (average 290 g/kg DM). However, starch content was lower than faba bean seeds (Hejdysz et al., 2016, Duc et al., 1999, Proskina and Cerina, 2017) (423 g/kg DM). SBM is a good source of CP (Council, 2012) (490 g/kg DM). However, FBP contained more CP (632.41 g/kg DM) compared to SBM. FBP had the highest CP source for broiler chickens followed by SBM and faba bean seed.

The major proteins in faba bean seeds are globulins with 80% of total storage proteins and consist of legumin and vicilin (Horstmann et al., 1999). Legumin proteins are high in SAA (methionine and cysteine) while vicilin proteins are rich in lysine (Warsame et al., 2018). On the other hand, the main proteins of SBM are glycinin (comparable with legumin) and β-conglycinin (comparable with vicilin) (Nishinari et al., 2014). Cysteine and methionine were the limiting AAs in faba bean protein concentrate and SBM, and lysine and methionine were the first limiting AAs for poultry (Gunawardena et al., 2010, Woyengo and Nyachoti, 2012). Faba bean protein concentrate had higher lysine (4.44% DM) and lower methionine (0.48% DM) (Gunawardena et

al., 2010) compared with SBM; lysine (3.30% DM) and methionine (0.66% DM) (Woyengo and Nyachoti, 2012). In this present study, FBP diets had higher lysine (20.10 g/kg DM) and lower methionine (8.85 g/kg DM) compared with SBM; lysine (18.60 g/kg DM) and methionine (8.90 g/kg DM).

5.2. Processes

In the pellet press, screw speed, capacity, and steam consumption differed in SBM and FBP diet to obtain high pellet quality. FBP diets contained finer particle size distribution compared with SBM diets (Figure 8). Therefore, feeder speed and capacity decreased comparing to SBM-P diet when FBP-P diet was producing. Conditioner temperature was kept at 81˚C for both diets. However, due to small particle size, lower capacity, and feeder speed, FBP-P diet exposed more friction in the die compared with SBM-P diet. As a result of high friction, the post pellet temperature of FBP-P (93˚C) was higher than SBM-P (89.2˚C). The temperature increasing between the conditioner and post pellet temperature was found to be similar with previous studies (Svihus et al., 2004, Svihus and Zimonja, 2011). The steam consumption was higher (29.5 kg/h) in SBM-P than FBP-P diet (19.6 kg/h). The reason for that can be different particle size distribution, protein structure, feeder speed, and capacity. Finer particle sizes have a larger surface therefore FBP-P diet could absorb steam better and reach 81˚C readily. SBM-P diet could absorb steam slower because of larger particle size distribution (Gilpin et al., 2002). FBP-P diet contained untreated proteins from both FBP and wheat. However, SBM-P diet contained untreated proteins from wheat but denatured proteins from SBM. Denatured proteins may absorb less water than untreated proteins because untreated proteins have higher water holding capacity due to the higher yield of hydrophilic portion (Abdollahi et al., 2013, Draganovic et al., 2014). Due to higher feeder speed and capacity, SBM-P diet had lower retention time in the conditioner and lower exposure to the pellet die compared with FBP-P. The parameters such as die specification,

conditioner temperature, motor load, steam pressure, total amperes of motors, and energy consumption were kept similar in SBM-P and FBP-P diets to obtain the same characteristics of pellets.

In the extrusion process, all parameters were not constant during SBM-E and FBP-E diet production. The reason for this could be that, SBM and FBP had different rheological properties. During production of both diets, conditioning temperature, feeder rate, screw configuration, temperature profile in different sections, drive power, SME, die specifications, and the number of knives were kept constant. On the other hand, both diets had different rheological properties during extrusion therefore some parameters such as steam and water addition in conditioner, calibration, screw speed, torque, die pressure, and knife speed were different. Calibration of SBM-E and FBP-E diet were different because they could have different particle size distribution and density. Addition of steam and water in conditioner were higher in FBP-E diet (10.2 and 17.5 kg/h) than in SBM-E diet (8.5 and 13 kg/h) subjected with screw speed (599 and 475 rpm) respectively. This can be explained by the flow property of FBP-E mash in extruder. SBM-E diet required less moisture addition than FBP-E diet for the desired flow property. The NDF content can affect viscosity of the extrudates negatively. FBP-E contained 130 g/kg DM of NDF compared with SBM-E 106.7 g/kg DM of NDF. As a result of lower screw speed in SBM-E, it yielded higher torque (43%) than FBP-E (34.6%). Die pressure was related with torque. Higher torque resulted in higher die pressure therefore SBM-E diet had higher pressure (30 bar) compared with FBP-E (24.5 bar). Expansion in extruder is closely related with die pressure therefore to equalize expansion, knife speed of FBP-E increased from 650 rpm to 678 rpm.

Pelleted diets (SBM-P and FBP-P) contained more moisture (95.7-83.7 g/kg respectively) compared with extruded diets (SBM-E and FBP-E) (27.5-37.5 g/kg respectively). It is likely that, during conditioning, added moisture was lower and also used different phases of water in pelleting than extrusion. SBM-P contained 29.5 kg/h steam, FBP-P 19.6 kg/h steam, SBM-E 8.5 kg/h steam

and 13 kg/h water, and FBP-E 10.2 kg/h steam and 17.5 kg/h water. Additionally, moisture in pelleted diets was lower because the cooler could remove 4-8% of moisture in the pellets. On the other hand, a dryer which was used to remove moisture in extruded pellets, could remove 15-20% of moisture in the pellets. Pelleted diets were cooled by the ambient air for 45 minutes however extruded diets were dried by dry air which was 110˚C in the 1st segment of the dryer and 65 ˚C in the 2nd segment of dryer for 3% belt speed (10 min). The moisture differences between pelleted and extruded diets could be caused by different moisture removing process and time of cooling and drying.

5.3. Physical quality of feed

Physical pellet quality is defined as pellet resistant against opposing disintegration and abrasion during conveying and pneumatic handling without breaking up or generating minimum proportion of fines (Cramer et al., 2003, Amerah et al., 2007, Abdollahi et al., 2013). The highest durability was obtained in the FBP-E diet and this could be due to moisture (27.7 kg/h) addition and containing raw proteins. SBM-P having the highest CP (251.6 g/kg DM), and containing treated proteins had 94.3% of PDI with 29.5 kg/h moisture addition. FBP-P had 93.7% of PDI with 19.6 kg/h moisture addition. SBM-E had the lowest durability (92.7%) with 21.5 kg/h moisture addition. However, correlation of this cannot be provided in this study because each diet had different moisture content. Increasing moisture content at optimum levels increases pellet durability therefore moisture acts as pellet binder (Abdollahi et al., 2012, Abdollahi et al., 2013). However, protein structure (raw or treated) is as important as moisture content. Abdollahi (2013) mentioned that denaturized proteins may provide a strong gelling property of feed and as a result, more durable pellets may be obtained. The result of the study by Wood (1987), showed that raw soybean pellets had higher physical quality than denatured proteins pellets because raw proteins increase the gel-forming property compared with treated proteins. FBP diets contained raw

proteins and therefore more proteins could be denatured during feed processes than SBM diets which have denatured proteins due to soybean meal production. SBM-P may have higher PDI than FBP-P due to SBM-P contained the highest CP in all diets.

The previous studies showed that hardness increases when expansion decreases (Lue et al., 1990, Hansen and Storebakken, 2007). This could be a reason for the differences in hardness for extruded and pelleted diets. Differences of hardness between diets produced with the same process were caused by NDF content, and addition of (58 g/kg) cellulose powder in FBP diets. Hansen and Storebakken (2007) observed that increasing cellulose inclusion also increased hardness. Because the cellulose molecule contains repeating glucose unit which has a three hydroxyl group and hydroxyl group provides strong hydrophilic properties such as water binding capacity (Boulos et al., 2000, Hansen and Storebakken, 2007). Therefore, the water binding capacity of cellulose improves the water holding capacity of diets (Hansen and Storebakken, 2007). Hardness of FBP-P diet was higher that SMB-P, whereas hardness of FBP-E diet was higher than SMB-E. The reason for this could be that FBP-P contained 26.2 g/kg DM more NDF compared with SBM-P. For extruded diets, FBP-E contained 23.3 g/kg DM more NDF compared with SBM-E.

Water stability test has been used to measure physical quality of aquatic feeds. Baeverfjord (2006) mentioned that extruded pellets have higher water stability, PDI, and absorb more water than pelleted pellets. Baeverfjord may compare extruded and pelleted fish feed which contains approximately 430 g/kg DM of CP, 335 g/kg DM of crude lipid, and 95 g/kg DM of starch. The water stability test measures how quickly the pellets disintegrate with mild physical stress (shaking) to disperse and/or dissolve in water (Baeverfjord et al., 2006). Thus, the added lipid in extruded pellet's pores should not be leaked before reaching fish digestive tract. In this study, the water stability of pelleted diets was higher than extruded diets. It may be caused by the difference in expansion rate and feed process parameters. Pelleted diets had no expansion and were

produced with low intensity. On the other hand, extruded diets had expansion and contain pores. Pores may act as coarse particles in pellets and cause weak points (Thomas et al., 1998, Svihus et al., 2004). Therefore, extruded diets were performed poor during the water stability test even though they contained high PDI results.

Particle size distribution was different between SBM and FBP diets due to the milling. In SBM diets, SBM ground by 1 mm hammer mill (coarse particles) and wheat ground by pin mill (fine particles). In FBP diets, both FBP and wheat ground by pin mill (fine particles) and cellulose powder was a finely ground ingredient. Due to different milling and protein sources, the particle size distribution may or may not affect hardness, durability, water stability, and expansion. However, the fine particles have a greater surface area and a greater surface area of feed particles are exposed to higher heat, moisture, pressure, and mechanical treatments than coarse particles during the feed process.

Physical pellet quality measurements such as hardness, durability, water stability, and expansion are used to measure pellet quality. High physical pellet quality form of feeds increases feed intake, and thus body weight and feed efficiency in broiler chickens (Svihus and Zimonja, 2011).

5.4. Broiler chicken performance

Feed intake, between days 17-23, 24-29, 1-29, was scientifically significant ($p<0.05$). During 17-23 days, protein source and process had no effect on feed intake. During 24-29 and 1-29 days, the process had no effect but protein source had an effect on feed intake. Physical pellet quality of FBP diets were similar to the SBM diet and therefore, the physical quality of the pellet could not affect the feed intake. FBP diets may be unpalatable for broiler chickens due to the α-galactosides and tannins. Porres (2002) reported that the antipalatable components (α-

galactosides and tannins) in legume seeds are related to low feed intake in rats. There is no study found to compare feed intake in broiler chickens fed with FBP, however there are studies found about broiler chickens fed with whole faba bean with low inclusion (50-250 g/kg) (Moschini et al., 2005, Diaz et al., 2006, Nalle et al., 2010, Gous, 2011) and broiler chickens consumed similar amounts of whole faba bean (mash faba bean, pelleted faba bean or extruded faba bean) diets and control diets (SBM based).

During 17-23, 24-29, and 1-29 days, body weight was scientifically significant ($p<0.05$). Protein source had an effect on body weight but process did not have any effect. Broiler chickens which consumed SBM diets had higher body weight compared with FBP diets. Feed intake depressed the body weight for FBP diets. However, whole faba bean (mash faba bean, pelleted faba bean or extruded faba bean) diets with low inclusion (50-250 g/kg) did not affect body weight compared with control diet (SBM based) (Moschini et al., 2005, Diaz et al., 2006, Nalle et al., 2010, Gous, 2011).

FCR was scientifically significant ($p<0.05$) during 17-23, 24-29, and 1-29 days. There was no correlation between protein source and process during 17-23 days. Broiler chickens consumed 702.1 g of FBP-E pellets and gained 553.6 g. On the other hand, FBP-P diet was consumed 749.6 g and broiler chickens gained 544.5 g. The chickens consumed lower amount FBP-E than FBP-P, yet they gained higher weight in FBP-E diet compared with FBP-P diet. During 17-23 days, the broiler chickens which were fed the SBM-E diet had more efficient FCR than SBM-P. During 24-29 and 1-29 days, process had no effect on FCR but protein source had an effect the FCR. The following studies (Moschini et al., 2005, Diaz et al., 2006, Nalle et al., 2010, Gous, 2011) found similar FCR with both low inclusion (50-250 g/kg) faba bean diets (mash faba bean, pelleted faba bean or extruded faba bean) and control diet (SBM based). Diaz (2006) observed that extruded faba bean diet had more efficient FCR than raw faba bean during 1-21, 1-42, and 22-42 days. The extrusion process can improve the nutritional value of faba bean and decrease or remove

ANFs such as trypsin inhibitors, lectins, phytic acid, and tannins (Alonso et al., 2000, Hejdysz et al., 2016). Diaz (2006) and in this study was found that extrusion process improves FCR.

5.5. Protein digestibility and trypsin enzyme activity

In UJ, process had scientifically significant ($p<0.05$) however in LJ, UI, and LI, protein source was scientifically significant ($p<0.05$). FBP-P (0.875) and FBP-E (0.902) had higher protein digestibility than SBM-P (0.813) and SBM-E (0.824). The particle size distribution differed between SBM and FBP. Lacassagne (1991) showed that the particle size distribution of raw faba bean had no effect on protein digestibility in broiler chickens. However, during pelleting and extrusion processes, steam and/or water were injected into the mash to increase moisture and temperature and the finer feed particles can be treated by steam more easily than coarse feed particles due to the surface area (Gilpin et al., 2002). The FBP diets with finer particle size, may reduce more heat-labile ANFs such as protease inhibitors, lectins, phytic acid, and tannins and increase NSP solubility more than SBM diets (Boroojeni et al., 2016). The previous studies (Alonso et al., 2000, Diaz et al., 2006, Hejdysz et al., 2016) showed that the extrusion process increased protein and starch digestibility via decreasing ANFs. In this study, similar result was found. FBP-E had higher protein digestibility than FBP-P and SBM-E also had higher protein digestibility than SBM-P. In UJ, pelleted diets can have higher protein digestibility because proteins in pelleted diets could contain weaker magnitude of disulfide bonds (Selle et al., 2012) than proteins in extruded diets due to lower temperature in pelleting process. In LJ, UI, and LI, FBP diets had higher protein digestibility than SBM diets. The ingredients of FBP diets contained more denatured proteins than SBM diets because SBM had excessive heat and moisture treatments during soybean meal production. Under those excessive treatments, soybean proteins may have stronger disulfite bonds which decrease the protein digestibility by restricting protease enzymes' activity (Selle et al., 2012).

The protein digestibility in the ileum of the broiler chicken was 88.8% of zero-tannin faba bean diets and decreased with tannin inclusion down to 80.8% (24 g/kg of tannins) (Ortiz et al., 1993). However, the apparent ileal digestibility of protein was found to be 84.4% in zero-tannin faba bean and 77.5% in conversional faba bean diets in broiler chickens (Woyengo and Nyachoti, 2012). FBP-E had higher ileum protein digestibility than that found by Ortiz (1993) and Woyengo and Nyachoti (2012) in broiler chickens. FBP-P had higher protein digestibility than raw zero-tannin faba bean (84.4%) and raw conversional faba bean (77.5%) diets (Woyengo and Nyachoti, 2012) but lower than raw zero-tannin faba (88.8%) (Ortiz et al., 1993). In grower pigs, faba bean protein (air-classified) (82.2%) had higher apparent ileal protein digestibility than soy protein concentrate (73.5%) because faba bean protein (air-classified) had smaller particle size than soy protein concentrate (Gunawardena et al., 2010). The particle size of feed could be more important in protein digestibility for pigs than poultry due to lack of gizzard. According to Gunawardena (2010), faba bean protein (air-classified) was specified as the superior protein source than soy protein concentrate for grower pigs.

No significant differences were found for trypsin enzyme activity. However, extruded diets had higher trypsin enzyme activity than the pelleted diets. The reduction of ANFs such as protease (trypsin and chymotrypsin) inhibitors, lectins, phytic acid, and tannins may increase the trypsin activity of extruded diets compared with pellet diets during feed processes. Protease (trypsin and chymotrypsin) inhibitors cause the inhibition of trypsin and chymotrypsin enzymes and as a result, the protein digestion and utilization decreases in broiler chickens (Gatel, 1994). Alonso (2000) found that the extrusion process removed trypsin and chymotrypsin inhibitors, lectins, phytic acid, and tannins in faba beans. Raw faba bean contained 4.47 IU/mg DM of trypsin inhibitor activity and 3.56 IU/mg DM of chymotrypsin inhibitor activity (Alonso et al., 2000). Trypsin inhibitor activity was found 4.50 mg/g as-is in faba bean protein fraction (Gunawardena et al., 2010). After the extrusion process, trypsin inhibitor activity decreased from 4.47 IU/mg DM to 0.05 IU/mg and

chymotrypsin inhibitor activity decreased from 3.56 IU/mg DM to 1.68 IU/mg DM in faba bean (Alonso et al., 2000). Hejdysz (2016) was also found that the extrusion process can decrease trypsin inhibitor activity 33-50% of different faba bean cultivars. The trypsin enzyme activity was higher in FBP diets (4.760 U/g chyme) than SBM diets (4.411 U/g chyme). The additional fiber, insoluble fiber, and NDF content increased the activity of trypsin and chymotrypsin enzyme in poultry (Bogułsawska-Tryk, 2005). FBP-P and FBP-E contained the additional cellulose powder and (134 and 130 g/kg DM) more NDF compared with SBM-P and SBM-E (107.8 and 106.7 g/kg DM). According to Kheravii (2018), the large and hard feed particles and fiber developed gizzards. A well-developed gizzard increases the release of cholecystokinin that stimulates pancreatic enzyme secretion (Kheravii et al., 2018). FBP diets contained 76.58 N of hardness and 94.58% of PDI compared with SBM diets (51.69 N of hardness and 93.5% of PDI). Therefore, the additional fiber, NDF content, and gizzard development may cause higher trypsin enzyme activity in FBP diets than SBM diets.

5.6. Anti-nutritional factors

Faba bean seeds contain different of ANFs such as protease inhibitors, lectins, tannins, phytic acid, NSP, and oligosaccharides which reduce nutrients digestion and absorption. Feed processing technology can remove or reduce heat-labile ANFs. Lectins are glycoproteins that bind trypsin and chymotrypsin enzymes and may cause hemolytic anemia in broiler chickens. Lectins are heat-labile compounds. Alonso (2000) was observed that raw faba bean contained 49.3 HU/mg DM of lectins activity however extruded faba bean contained 0.2 HU/mg DM of lectins activity. Extrusion process is about to inhibit lectins activity. Gatel (1994) mentioned that the pellet press was not an effective process to reduce lectins. In this experiment, during the pelleting process, the added steam was 19.6 kg/h and conditioning temperature was 81˚C therefore, the

combination of heat and moisture could remove lectins from FBP-P diet as Jaffé and Vega Lette (1968) mentioned.

Tannins are one of the major ANF in faba bean. Tannins can form with proteins, cellulose, hemicellulose, pectins, nucleic acids, steroids, alkaloids, and saponins and those complexes can become indigestible for broiler chickens. The main anti-nutritional effect of tannins in broiler chicken is depressed the protein and AA digestibility (Gatel, 1994). They are mainly located in the seed coat (2.7 g/kg) however small amount of tannins are found in cotyledon (0.3 g/kg) (Ortiz et al., 1993). Dehulling and extrusion are two effective process to reduce tannins content in faba bean seeds. According to Alonso (2000), dehulling decreased 92.3% of tannins content from 1.95 (g eq cat kt-1 DM) to 0.15 (g eq cat kt-1 DM) and extrusion reduced 54.5% of tannins content from 1.95 (g eq cat kt-1 DM) to 0.89 (g eq cat kt-1 DM) in faba bean. Using dehulled faba bean instead of whole faba bean in high tannin containing variety can increase nitrogen digestibility up to 7% (Gatel, 1994). In this study, FBP was dehulling before air classification process and pelleting and extrusion processes were used.

Phytic acid or phytate is the main storage form of phosphate in faba bean seeds. It is also ANF for broiler chickens. Phytic acid forms complexes with minerals (phosphorus, calcium, zinc, magnesium, iron, and copper), proteins, carbohydrate (starch), lipids, and protease enzymes (pepsin and trypsin) which are not bioavailable for broiler chickens. Phytic acid has a stable structure however Alonso (2000) observed that the extrusion process can remove phytic acid from 21.7 g/kg DM to 15.9 g/kg DM by 37% in faba bean. In this study, large amount of rapeseed oil (76 g/kg as-fed) was used in all diets without phytase enzyme (Table 7). Matyka (1990) and Leeson (1993) observed that lipid and phytate can form soap in the digestive tract of chicken and phytate utilization was inhibited by lipid. Therefore, lipid digestibility decreased and morphology of digestive tract could affect negatively from phytate-lipid soap.

The RFOs such as sucrose, raffinose, starchyose, verbascose, and ajugose are ANFs because α-galactosidic linkages of RFOs are not digestible due to the lack of enzyme deficiency for broiler chickens. However, the RFOs are digestible for microorganism in ceca and large intestine which also cause flatulence (Baucells et al., 2000). Flatulence may cause the lower nutritional utilization (Alonso et al., 2001). Choct (2010) observed that RFOs increase the viscosity of digesta content, hydrogen production (microbial fermentation), and diarrhea in broiler chickens. However, the feed process such as extrusion can reduce RFOs by 21% in kidney beans. In this experiment, RFOs may increase the viscosity of digesta content and higher retention time of digesta content in digestive tract may not affect protein digestibility in small intestine. However, the higher retention time of digesta content in lower part of intestine may cause loss of appetite in broiler chicken and may induce lower feed intake in FBP diets. It is similar of the hypothesis that slow passage rate of digesta content limits the feed intake of broiler chickens (Svihus et al., 2002, Amerah et al., 2007).

NSP may be more an ANF than a nutritional factor for broiler chickens. Soluble NSP may be hydrolyzed 80-90% by microbial fermentation but insoluble NSP is not digestible for chickens (Carré et al., 1995). Insoluble NSP does not create direct problems related to digestion except for being indigestible by broiler chickens. On the other hand, soluble NSP can create secondary problems by affecting the morphology of the digestive tract. In the beginning part of the caeca, soluble NSP can begin to increase the viscosity of the digesta content. As a result of this, soluble NSP may cause morphological changes in the intestinal tract, disturb the secretion of endogenous enzymes and bile acids, and reduce the digestibility of nutrients (Jamroz et al., 2002, Choct et al., 2010). Total NSP of raw soybean (159.6 g/kg DM) (Bueno et al., 2018) was the highest followed by SBM (157 g/kg DM) (Knudsen, 1997) and FBP (109.64 g/kg DM). Raw soybean contained 36.7 g/kg DM of soluble NSP and 122.9 g/kg DM of insoluble NSP. SBM on the other hand contained 64 g/kg DM of soluble NSP and 93 g/kg DM of insoluble NSP. During SBM production,

the grinding, heat, moisture, and pressure treatments were applied to the raw soybean (Wright, 1981). Due to these treatments, insoluble NSP content had shifted to soluble NSP. According to De Vries (2012), hydrothermal processing such as extrusion and pelleting can increase the solubility of NSP by grinding, heat and pressure. The solubility of NSP increases during the feed process which causes breakage in covalent and non-covalent bonds between the cell wall components and polysaccharides as well as loss of side chains and partial degradation of NSP polymers (De Vries et al., 2012). As SBM was produced, the solubility of uronic acid increased 14.9 g/kg DM, galactose 5.7 g/kg DM, arabinose 4.3 g/kg DM, mannose 3.2 g/kg DM, rhamnose 1 g/kg DM, xylose 0.4 g/kg DM, and glucose 2.2 g/kg DM decreased (Knudsen, 1997, Bueno et al., 2018). There is no study describing how a hydrothermal process affects the solubility of NSP in *Vicia faba*. However, different hydrothermal processes, types of NSP, bonds between polysaccharides, and other cell wall components have an effect on the solubility of NSP. In this study, SMB contained a higher amount of soluble NSP than untreated FBP (pelleted and extruded NSP content of FBP is unknown). The neutral sugars content between SBM and FBP were different and their effects in the digestive tract could be different. The specific type of neutral sugar may cause higher anti-nutritional effect than the amount of soluble NSP. The body weights of broiler chickens which were fed with the FBP diet were lower than SMB diets. Apart from the feed intake, poor lipid digestibility may cause lower body weight in FBP diets because the solubility of NSP and digesta viscosity inhibited lipid digestibility more than other nutrient digestibility in raw and extruded diets (Dänicke et al., 1997, Son and Ravindran, 2012). FBP was ground finely by the pin mill and SBM were ground by the hammer mill (1 mm screen size) therefore the finer particles of FBP diets were more open for heat, moisture, pressure, and enzymes (added to the diet) treatments than SBM diets. As a result of treatments during feed processes, the insoluble NSP content may shift to the soluble NSP. The soluble NSP and RFOs of FBP diets may increase the viscosity of digesta content in the digestive tract and may depress the feed intake.

6. CONCLUSION

Protein digestibility of extruded and pelleted FBP diets was higher than for the SBM diets. The protein digestibility is determined by the source of protein, rather than feed process techniques. Even though protein digestibility was higher in FBP diets, bird performances such as body weight, feed intake, and FCR were lower for the broiler chickens fed with FBP diets. No negative physical qualities were detected in the feed, therefore the limitation on broiler chicken performances was unknown. However, feed production techniques such as grinding, dehulling, conditioning, pelleting, and extrusion can eliminate the activity of ANFs such as protease inhibitors, lectins, tannins, and phytic acid. However, the effect of feed processes on RFOs and NSP, and the behavior of RFOs and NSP in the broiler chicken digestive tract can be investigated in further studies.

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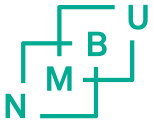

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