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The effect of expanded and extruded process on pellets physical properties and *in sacco* rumen degradability

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Declaration

I, Huimin Li, warrant that this thesis is a result of my original experiment and findings. I have read the University current ethics guidelines. Sources of information other than my own have been acknowledged and a reference list has been appended. This work has not been previously submitted to any other university for the award of any type of academic degree.

Signature…….

Date……16. 8. 2021…….

Acknowledgements

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Summary

Rumen degradability kinetics was investigated for concentrates with different physical functional properties. Three diets with SBM (30%) and barley (70%) were produced by ether expanded press process (Con) or by an extrusion process using two distinct procedures giving pellets with either high density (HD) or Low density (LD). The procedures in grinding, mixing and cooling were the same. Con was produced in expander where its cooking time was only seconds. LD and HD were produced in the extrusion process with the temperature of 120 and 90 ^{o}C respectively. Feed physical properties of three diet pellets, including durability, hardness, stability, density were detected. Ruminal degradation of dry matter was conducted by *in sacco* methods. In total, durability in the extrusion process group, LD (95.8%) and HD (99%), showed higher than expanding process (92%). Concentrate produced in HD has the highest hardness (112.01 N). LD group is floating with low specific density (0.70 g/ml) and low bulk density (412.3 g/L). Both HD and Con have high sinking velocity with similar high specific density (SD) and high Bulk density. Change of specific SD in rumen fluid for HD group is less, for LD is large but still not over 1.0 g/ml. The extruded concentrate showed high fluid stability (WSI) than the expanded concentrate. The extrusion process can be used to alter the degradation rate of concentrate DM in dairy cows. It is possible to shift the digestion of nutrients from rumen to the small intestine. Extruded concentrate reduced effective DM degradability (ED) *in sacco* compared to the expanded concentrate. The reduction of ED was most pronounced for HD concentrate with the highest WSI and highest density. The pattern of DM degradation *in sacco* was similar between pelleted and ground concentrate.

Key works: extrusion process, physical properties of concentrate, rumen degradation, *in sacco*.

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1 Introduction

High production dairy cows have a high requirement of protein and starch to meet their needs for milk production. Two approaches used to increase the availability of nutrients are 1) increased quantity and ratio of end products from rumen fermentation and 2) increased rumen escape of nutrients for absorption in the small intestine (McCarthy et al., 1989). For approach 1, higher energy density is commonly provided using starch as the main nutrient. Starch is fermented in the rumen and converted into volatile fatty acids that are absorbed and metabolized as energy-yielding substrates. However, a high amount of rapidly fermentable starch is likely to result in rumen acidosis, which reduces rumen digestion and is potential to increase the incidence of metabolic diseases like ketosis and lameness (Nikkhah, 2012). Moreover, the presence of ruminal microorganisms provides the opportunity for ruminants to convert cellulose into nutrients that meet the animal's needs. However, it is also the main factor resulting inefficient use of dietary N. It has been shown that Milk N efficiency (MNE), an index to assess the efficiency of conversion of diary N into milk, in dairy cows range from 20 to 35%. In other words, 65 to 80% of diary N is excreted in feces and urine, causing potential environmental pollution (Mutsvangwa, 2011). Considering about pig production, N-retention rate (proportion of intake) is 41 - 68% in weaner pigs and 42 - 56% in grower pigs (KIM & PLUSKE, 2016). Therefore, compared with approach 1, the nutrients escaping rumen fermentation into the small intestine are preferred to improve nutrient efficiency. In ruminants, together with the choice of ingredients, feed processing technology is commonly used to manipulate digestion where the rate of rumen degradation is usually targeted. For example, steam pelleting can increase rumen undegraded protein (RUP) and decrease rumen undegraded starch (RUS) due to the heat involved.

Similarly, conventional pellets treated with the annular gap expander have been shown to increase RUP and RUS (Ljøkjel et al., 2003b). However, since rumen digestion is the net result of the rate of degradation and the rate of passage, altering the passage rate could be an alternate approach to shift the site for nutrient digestion from rumen to the small intestine. In order to alter the rate of passage, the density of feed particles is critical (Dufreneix et al., 2019). Low-density particles with low sinking velocity will float in the rumen, but particles with optimal high-density and high sinking velocity will sediment. These high-density particles will have more probability to escape rumen and be digested in the small intestine.

Recently, it has been shown that physical properties of feed pellets like density, sinking velocity, and fluid stability could play an essential role in the passage of feed particles (Khan, 2021). Feed pellets with optimal density and sinking velocity may decrease rumen retention time and help feed particles

to escape from microbial degradation and thus provide more protein and starch that can enter the small intestine. Beside density, high pellet stability in the ruminal fluid is also essential to avoid the pellet disintegrating early and, therefore crucial for maintaining density properties of pellets. In addition, high fluid stability of pellets will decrease the rate of rumen degradation of feed particles and hence nutrients. These pellet physical properties are possibly be achieved by changing the feed production process. However, for ruminants, feed pellets are commonly produced by conventional steam pelleting in which physical properties of pellets like density and fluid stability are hard to control. Compared with conventional pelleting, extrusion pelleting, commonly used in the fish industry, is available to control the physical properties of pellets and thus provides the potential to be also used for ruminants concentrate feeds.

Determination of physical properties of feed pellets for ruminants is usually limited to durability and hardness. Except for Khan (2021), density, sinking velocity, and fluid stability of feed pellets for ruminants have not been determined before and need more investigation. Apart from physical properties, determining the degradation rate of pellets with exceptional physical properties is essential. Generally, the rate of degradation of a feed is determined by *in sacco* technique as described by Åkerlind et al., 2011. In this technique, samples are ground before incubation in the rumen. However, grinding feed pellets will lose their physical properties. Hence, a more detailed investigation of *in sacco* procedure with the ground and intact pellets is needed.

The current study aimed to investigate how conventional pelleting and extrusion pelleting processes may affect the physical properties and dry matter degradation of feed pellets. It was hypothesised that 1) as compared to expander treated conventional pellets, extruded pellets will have high fluid stability; 2) pellets with high fluid stability and high density will have lower dry matter degradation measured with the *in sacco* method; and 3) intact pellets will have lower *in sacco* degradation parameters than ground pellets.

2. Theory

2.1 Ruminant physiology

Ruminant has evolved a set of stomachs capable of digesting fibrous materials like cellulose and hemicellulose (Dijkstra et al., 2005). As shown in figure 1, the stomach of the ruminant is composed of the rumen, the reticulum, the omasum, and the abomasum, where the three first represents the fore-stomachs and the last the real stomach comparable to the stomach of monogastric. Among the stomachs, the rumen and the reticulum account for around 85% of the total capacity (Dijkstra et al., 2005) and since they are closely linked, commonly named the reticulorumen.

Reticulorumen

Feed enters the reticulorumen through the oesophagus after chewing and swallowing. In the reticulorumen, microorganisms ferment the feed nutrients, mainly carbohydrates, and the main products are volatile fatty acids (VFA) and microbial protein. Most of the VFA produced is absorbed through the rumen wall and transported to the liver via through hepatic portal vein. Volatile fatty acids mainly involve acetic, propionic, and butyric acid. Acetic acid is in the greatest quantity and yields around 20-50 moles per day, usually constituting a molar proportion of 50 to 70% of the total VFA depending on diet composition (Dijkstra et al., 2005). Propionic acid is usually produced in quantities around one-third of the molar proportion of acetic acid. Butyric acid usually accounts for only around 10 % of the total molar production of VFA (Dijkstra et al., 2005). There are also a few valeric, iso-valeric, and iso-butyric acids produced. These VFA's constitute normally less than 5% of total VFA, and the two iso acids originate from microbial degradation of amino acids (Dijkstra et al., 2005). Acetate is used as energy in the ruminal tissue or taking part as energy and building blocks in fat synthesis including milk fat. Propionate is utilized together with amino acids for gluconeogenesis in the liver. The glucose released is used to synthesize lactose in the mammary gland and synthesize energy in the placenta. Most Butyric acid is metabolized in the rumen wall to 3-hydroxy-butyrate, used for energy and fat synthesis (Dijkstra et al., 2005).

Large quantities of feed protein and urea are recycled via the saliva and metabolized to ammonia. Microorganisms utilize amino acids, small peptides and a large quantity of ammonia for microbial protein synthesis. When the microbes are flushed out of the rumen, they will together with rumen escape dietary proteins and be digested and absorbed as amino acids in the small intestine. Usually, microbial proteins account for the dominating proportion of absorbed amino acids. Ammonia not utilized by the microorganism will be absorbed through the rumen wall and transported by the blood

to the liver. Here the ammonia is converted to urea, either recycled to the rumen and metabolized back to ammonia or excreting in the form of urea in urine (Dijkstra et al., 2005).

Other parts of the digestive tract

Digesta enter the omasum via the reticulo-omasal orifice. The omasum is filled with 100 tissue leaves where water, ammonia, VFA, and inorganic electrolytes are absorbed (Dijkstra et al., 2005). Digesta entre the abomasum from the omasum. The function of the abomasum is similar to the monogastric stomach in which acid and enzymes are secreted and mixed with digesta. Dietary amino acids and other dietary digestible nutrients escaping microbial fermentation are together with microbial protein and other microbial nutrients digested in the abomasum and the small intestine. After passing through the small intestine, the digesta moves into the large intestine where it in the caecum and proximal colon are subjected to both peristaltic and antiperistaltic contractions, mixing and moving the digesta towards the distal colon (Dijkstra et al., 2005). There is additional VFA production and absorption in the large intestine, but the primary function is probably water absorption (Dijkstra et al., 2005).

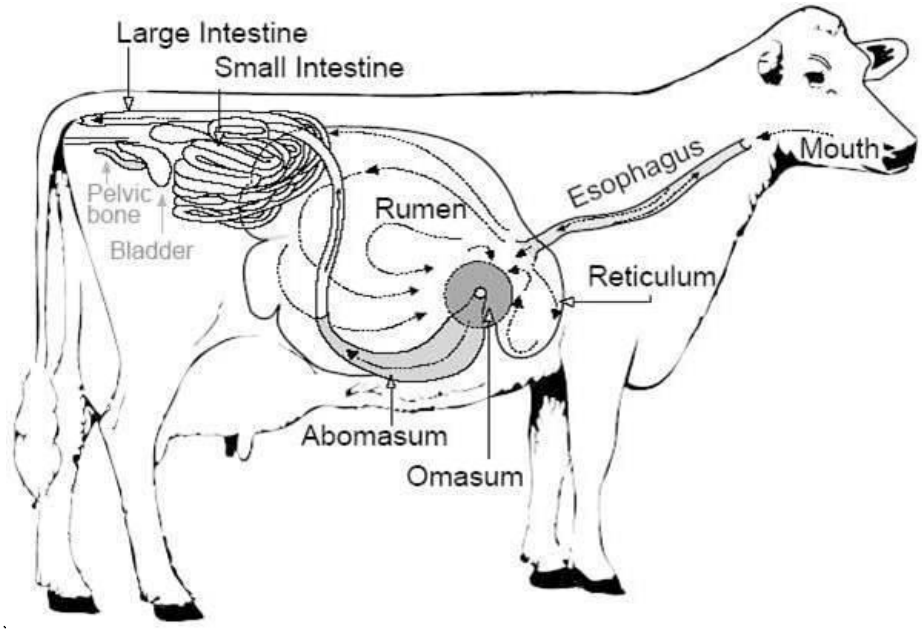

Figure 1. The digestive system of cattle (Böhnlein, 2007)

2.2 Rumen digestion dynamics: the rate of degradation and rate of passage

Digestion in the rumen includes two main components: degradation and passage and is determined through the fractional rates of degradation and passage. Feed type and quality together with feeding level are dominant factors influencing degradation rate and passage rate (Ehle, 1984; Offner et al.,

2003). Feed dry matter is including soluble material and insoluble material. Soluble material is regarded as digested instantly and has a passage rate equal to the rate of rumen liquid passage. The insoluble component is degraded over time, and the passage rate differs related to the intrinsic properties of the feed particles. For instance, ground concentrates and forage pass through rumen more quickly than large fibre particles since small, dense particles sink and leave the rumen more quickly than large particles that are floating and ruminated, reducing rumen retention time and thereby reducing the rate of passage (Ehle, 1984).

Rate of passage affects nutrition utilization since it decides the available time for nutrient degradation and absorption (Ehle, 1984). For ruminant, the passage of digesta involve selective retention, mixing, segregation, escape from attacking by rumen microorganism before passing into the small intestine. It has been shown that increased feeding level will decrease apparent diet digestibility but increase the passage rate. As shown in Okine & Mathison (1991), if feed intake of nurture detergent fibre (NDF) increases from 7.0 to 11.3 kg/d, NDF passage rate increase from 2.2 to 2.4 %/h, and its mean retention time decrease from 45.5 to 38.5 h. Finally, its digestion rate decreases from 3.05 to 2.70 %/h. Feed physical properties also affect passage rate, in which density and particles sizes are the main factors. According to Kaske et al. (1992), four times as many particles with a 1.44g/ml density pass through the sheep omasum than particles with a low density (0.92 or 1.03 g/ml). Small particles (1 mm) were passed through three to ten times than large particles (10 and 20 mm).

Some research underlined how the rate of degradation could be affected by feedstuff. Starch represented a large proportion of diet can affect degradation rate to a large extend. The rate of starch degradation ranges from 0.05 h^{-1} for corn and sorghum to more than 0.30 h^{-1} for wheat. As shown in figure 2, the content of rumen degraded starch and undegraded starch varies in different feedstuff, in which degraded starch is higher in wheat whereas lower in toasted peas (Offner et al., 2003). It is similar to protein degradation. It was investigated that escaping soluble protein from rumen ranged from 0.15 to 0.56 h-1 in concentrate and from 0.25 to 0.33 h-1 in fresh herbage. The linseed cake shown a slow degradation rate since the high level of mucilage in linseed cake may protect part of the protein from degradation (Hedqvist & Udén, 2006).

Feed processing is also a factor that affects the rate of degradation. It has been investigated that thermal treatment largely increases starch degradation in the case of slowly degraded material. Gelatinization during the heat process led to chemical and physical changes in the starch granule. The disruption of hydrogen bonds and water absorption resulted in microbial degradation of the granules. Whereas in an exceptional case, heat treatment may negatively affect starch degradation. For

example, low starch degradation occurs by the formation of complexes between starch and protein, and the soluble fraction of starch and protein decreases (Offner et al., 2003).

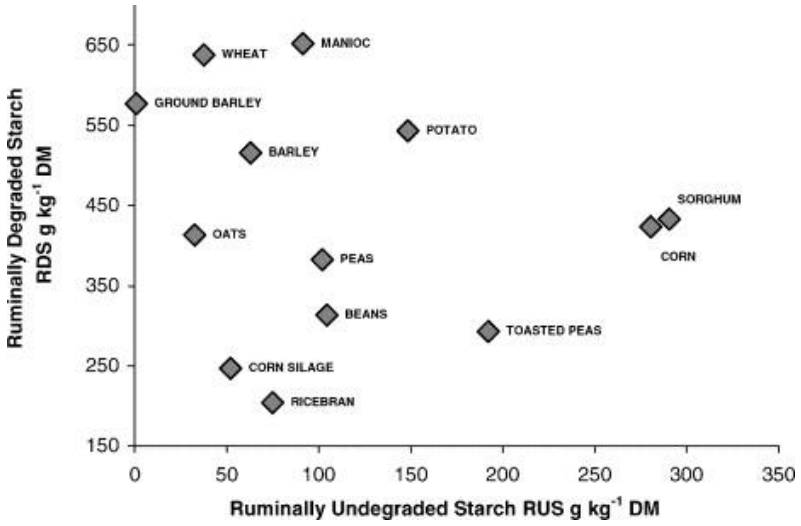

Figure 2. Contents of ruminal degraded and undegraded starch (Offner et al., 2003).

2.3 Feed processing

Feed processing includes several steps, including grinding, conditioning, and various methods for pelleting. In the following, some aspects of these methods are described.

Grinding

Grinding is a technology to reduce particle size. Particle size reduction has important effects such as an increased surface area for enzyme action, improved mixability of ingredients, increased particle homogeneity and elimination of germination ability.

The most common type of grinder is the hammer mill. Several rows of rectangular metal plates (the hammers) are attached to a shaft rotating at a very high speed in hammer mills. The feed ingredients fall into the grinding chamber from the inlet, and the ingredients are crushed by the hammer tip, collisions with the walls of the grinding chamber, and impact between particles. Properly sized material passes through a sieve following an air flow, whereas the material with a large particle size is retained for further grinding.

Another less frequently used mill is the roller mill. The roller mill comprises two or more rolls that crush the feed material as it passes through between the rolls. In each pair of rolls, one roll is fixed, whereas the other can be moved to regulate the gap distance (Deaton et al., 1989). In addition to gap

distance, particle size can be regulated by roller speed, the direction of rotation, roller corrugation and diameter of the rolls. Moreover, intrinsic properties of the feed material like water content influences particle size (Arnold & Schepers, 2004).

Conditioning

Conditioning is a process where steam is supplied and mixed with the feed ingredients. Ultimately the feed is preheated and becomes easier to shape. The conditioner consists of a cylindrical chamber with a rotating screw in the centre. Raw ingredients enter, the steam is supplied through nozzles as the screw rotates directing the feed from inlet to outlet, mixing feed and steam before the feed mash is pushed into the pellet press. The retention time in the conditioner can be adjusted from less than 20 seconds to over 60 seconds by changing the feeder rate or paddle angle of the screw. During the conditioning process, higher temperature and long cooking time both increase starch gelatinization, with increasing time resulting in a linear increase in percentage starch gelatinization (Lewis et al., 2015).

Pelleting process

Pelleting has become one of the most common feed production processes for ruminants, pigs, and broilers. It has plenty of benefits like increased density, more accessible transport, reduced dust production, improved hygiene of feed, increased feed intake of animals, improved nutrient digestibility and elimination of feed segregation. Feed pellets can be divided into two types, i.e., conventional pellets and extruded pellets. Conventional pellets can be further divided into ordinary steam pellets, and expander treated pellets. All these pellet production processes are described below.

Steam pelleting

During steam pelleting, conventional pellet presses are used to shape feeds. Several different pellet press can be found as shown in figure 3, but their mechanics is same. The pellet press is composed of a die and a pair of press rolls rolling along the inner surface, pushing the feed material through the die, which is a thick metal plate with plenty of holes. The thickness of the plate is different from 45 to more than 100 mm, and the diameter of the holes may vary between 1 and 20 mm depending on the feed requirement. The pellet press is located under the outlet of the conditioner, and the feed mash falls into the pellet press chamber by gravity. Then feed mash is pushed through the holes of the die by rolls. During this process, the friction between feed and metal and between different metal parts

results in temperature and pressure increased. Therefore, starch gelatinization and protein degradation occur and make different ingredients glue together. A knife attached to the die may cut the pellet into the desired length after feed mash is forced through the die.

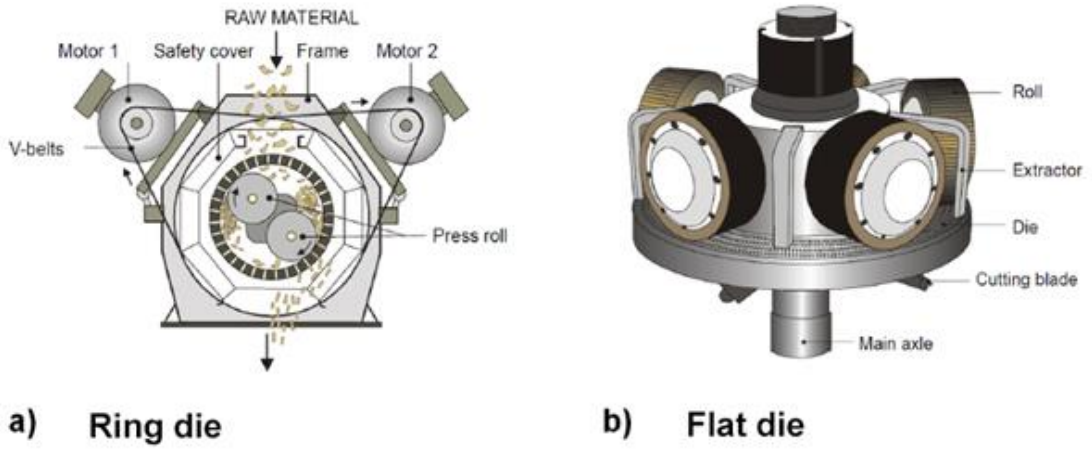

Figure 3. Pellet press with ring die and flat die (Stelte et al., 2012)

Expander pelleting

The expander can be considered as a type of conditioner where pressure is applied. The most common expander type is the annular gap expander as shown in Figure 4, which consists of a barrel, axis inside the barrel, and a cone-shaped head. The expander axis has a screw configuration, which increases friction and thus more effectively forcing the feed through the barrel. The retention time is short, usually between 2-10 seconds. A cone-shaped head restricts the opening of the outlet, and pressure is generated and can be changed by varying restrictions of the outlet opening. Pressure is usually between 20 and 30 bar. The temperature may reach between 70 °C and 130 °C depending on the restriction of the outlet opening.

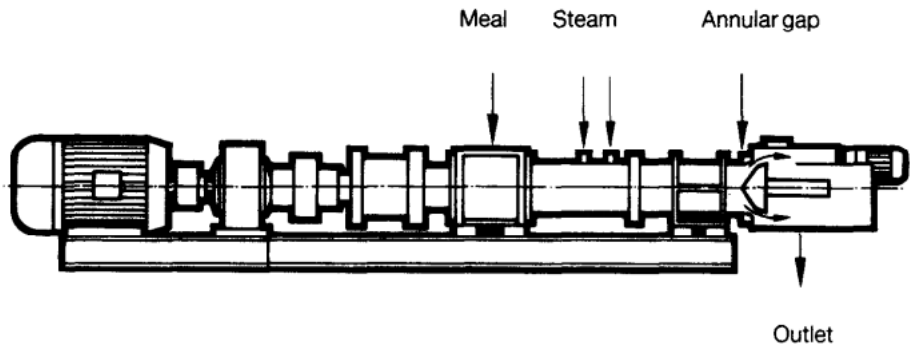

Figure 4. Annular gap expander (Audet, 1995).

Extruder pelleting

As the feed mash leaves the conditioner, it enters the extruder, as shown in Figure 5. The extruder is composed of one/two screws with adjustable configuration, and a metal chamber covered the screw and a die. The purpose of the first section of the extruder barrel is to transfer mash away from the inlet zone and into the cooking zone. The compression of the screw is increased in the cooking zone, and in this stage, steam or extra water is added and mixed with feed mash. The temperature of the mash is rapidly increased in this stage. The moisture addition and compression force of the screw configuration will result in a final pressure prior to the extruder die of 34.45 to 37.49 Bar, a temperature of 125 to 150 °C, and water content of 23 to 28% (Rokey et al.,2010). When mash passes through the die, it usually expands because of a sudden drop in atmosphere pressure (Ye et al., 2018). The knives cut off the pellet. Both expander and extruder are applied with high pressure and high temperature. Compared with the expander, the extruder has a long cooking time to utilize more moisture and friction to reach a higher temperature. Therefore, a higher degree of starch gelatinization and protein denaturation occurs in this process which improves the nutritional and technical quality of extruded pellets than expander pelleting.

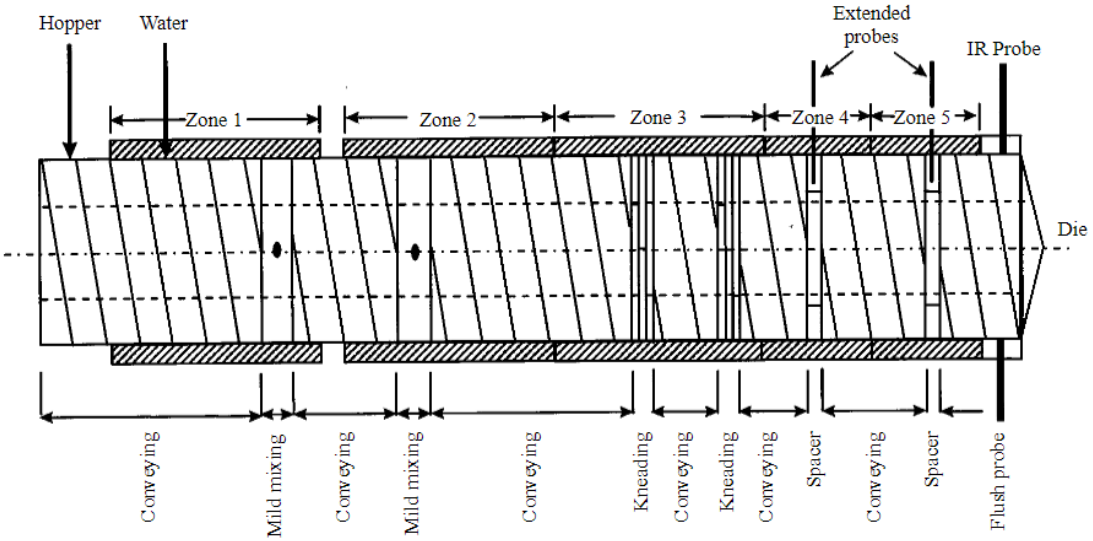

Figure 5. Schematic diagram of screw configuration (Godavarti & Karwe, 1997).

2.4 The effect of thermal processing on chemical change

Starch gelatinization

Starch gelatinization is a process from water absorption to the disintegration of starch granules. Starch granules swell after absorbing water. During swelling, almost all amyloses will leach out. During this

process, starch transfers from a crystalline structure to an amorphous state. However, limited water content and low temperature only allow a small extend of swelling. At present of sufficient water and temperature, the crystalline regions are rapidly and irreversibly broken down, and gelatinization is initiated. When pressure is up to 200 Mpa, gelatinization can take place at a lower temperature. If pressure is high enough, gelatinization can occur at room temperature (Stolt et al., 2000). Gelatinization is also affected by the ratio of amylose to amylopectin. Some research shows that extruded high-amylose maize starch presents crystallinity, while low-amylose maize starch becomes amorphous after extrusion. It also shows that the crystallinity of high-amylose maize starch decreased with increased moisture (Ye et al., 2018).

The degree of gelatinization is different among steam pelleting, expanding, and extrusion. It has been shown that the starch gelatinization in steam pelleting is only 1-19% but higher than 20% in expander pelleting due to high moisture content and pressure input. However, gelatinization during the extrusion process can be up to 100% since high moisture, high pressure, high temperature, and long cooking time are applied in this process(Svihus et al., 2005). It is also shown that starch from oats being easier to gelatinise than wheat and maize.

Protein denaturation

Protein is folded with a four-level structure, including primary, secondary, tertiary, and quaternary. Globular protein, as a storage protein in cereal, its tertiary structures determine the form of the molecule. And this structure is formed by non-covalent bonds and some sulphur bonds between secondary structures. Protein structure is unfolded easily when exposed to heat, and the presence of water may accelerate this process since the tertiary structure is not stable when suffering from heating. Changes occurred in these four structures are usually referred to as protein denaturation. With excess amounts of water, the extend of protein denaturation increases as temperature increases. The process of protein denaturation depends on the temperature applied, the processing time, and the moisture content during the process (Goelema et al., 1999).

2.5 The effect of thermal processing on animal and nutrition

Starch digestion and absorption rate are essential for managing metabolic disorders since the high intake of starch increase the risk for rumen acidosis, loss of appetite, laminitis, and health problems (Ljøkjel et al., 2003b).

According to Svihus et al. (2005), ruminal degradation of starch in maize and sorghum is higher by treated with extrusion as compared with ordinary steam pelleting. This would be expected since the

extrusion process involves more heat and long processing time, which caused a greater starch gelatinisation. However, Chapoutot and Sauvant (1997) conclude that extrusion decreased *in sacco* degradation rate 41% for crude protein for pea/rapeseed blends. In the study of Ljøkjel et al. (2003b), thermal treatment largely increase starch effective degradability in the slowly-degrading starch feedstuff. In the case of maize, steam flaking increased effective degradability up to 0.85 kg kg $^{-1}$. Whereas for the rapidly starch feedstuffs like barley and wheat, expanding and extrusion process has a negative effect on effective rumen degradability. It has also been shown that the relationship between temperature and effective rumen degradability is a decrease in effective rumen degradability of 0.016 kg kg $^{-1}$ with an increase of 10 ◦C

Protein solubility and protein degradation in the rumen are decreased as applied with heat treatment, protecting the protein from microbial attack (Goelema et al., 1999). According to Walhain et al. (1992), extrusion of peas did not significantly affect dry matter effective degradability but decrease crude protein effective degradability (88.3% vs. 65.5% at an outflow rate of 0.06 h^{-1}). Other research also shows that the extrusion of lupin seeds results in a 72% increase in protein flow to the duodenum of dairy cows (Rémond et al., 2003).

2.6 The effect of thermal-process on pellet physical properties

The feed mash will become viscous during the heating process due to starch gelatinization and protein denaturation. This viscosity contributes to binding of different ingredient in the feed and thus affect the physical quality of pellets. The combined effect of high temperature, moisture, pressure and shearing during the extrusion process improves starch gelatinization. Gelatinized starch adhere particles together and result in the high durability and hardness (Kaliyan & Vance Morey, 2009). Besides starch gelatinization, protein also can be used as a binder. As shown in Kaliyan and Vance Morey (2009), proteins from cereal grains with dough-forming capabilities such as wheat, barley, and soybean meal suffer from combined effects of thermal and shearing, resulting in protein denaturation. Thereby it has a positive effect on pellet durability. In addition, fibre is another factor to explain increased strength in pellets. It has been shown that durability increase from 93.8 to 97.4% if feed increase fibre content from 4.4 to 14.0% (Kaliyan & Vance Morey, 2009). Water-soluble fibre during extrusion may increase the viscosity of feed. The soluble fibres combined with water is viscous and adhere to the surfaces of solid particles to generate strong bonds. Water-insoluble fiber negatively affects pellet strength due to its stiffness and elasticity properties (Kaliyan & Vance Morey, 2009).

The moisture level is the dominant factor for extrudate density (Draganovic et al., 2011). A pilot extrusion trial shows that increased moisture content and die temperature may decrease pellet bulk

density and increase pellet floating possibility. According to Sharma and Gujral (2013), the high bulk density pellet is produced during extrusion at low temperature and high moisture, and high expansion pellet is produced at low temperature and low moisture extrusion (Sharma & Gujral, 2013). When steam is added, pellets exhibit different properties since starch gelatinization and protein denaturation vary with different water level addition. As shown in table 1, pellet durability is increased with the increasing amount of steam (Thomas et al., 1997).

Table 1. The effect of steam inclusion level on pellet durability (Thomas et al., 1997).

Steam		Durability
Pressure (kPa)	Added (kg t^{-1})	(%)
620.7	23.0	93.5
620.7	33.0	96.5
0	0.0	79.1
620.7	26.4	90.6
620.7	35.5	93.8
0	0.0	69.5

2.7 Methodologies

Evaluation of feed physical properties

Hardness

Hardness is a critical physical quality parameter of feed pellets and is defined as the maximum force needed to break a pellet(Thomas & van der Poel, 1996). Hardness can be commonly measured by a Kahl tester. This method simulates the force on pellets during storage in bins or silos and pellets crushing between animal teeth. One pellet is placed between two steel knobs, pressure is applied in the form of a spring, and the force is produced to crash the pellet (Thomas & van der Poel, 1996). Most reports test pellet in laying, and the requirement of force to crush the pellet will increase with pellet length if the test is carried out in standing (SØRensen, 2012).

Durability

Durability is another physical quality parameter of feed pellets, measured as the amount of fines returned from a batch of feed pellets under standard conditions (Thomas & van der Poel, 1996). Testing of pellet durability mimics the force on pellet taking place during filling of bins, during transportation from the feed producer to the farm, and during distribution in the feeding system at the farm. Pellets with high durability produce fewer fines (SØRensen, 2012).

Holmen durability tester is a common method to test durability. In this method, a 100 g pellet sample is transported around a closed circuit using air. After a standardized time of 30 to 120 s, pellets are sieved in a sieve with a sieve opening of 80% of the pellet diameter. Finally, the durability is expressed as PDI that is calculated as the mass of the pellets retained on the sieve divided by the total mass of pellets (SØRensen, 2012).

Density

Bulk density is defined as a feed mass per unit volume of space that the feed occupies (Khater et al., 2014). It is an important factor to control floatability or sinking velocity of pellets. Bulk density mainly depends on the degree of expansion during extrusion. A floating pellet is more expanded and its bulk density is low (SØRensen, 2012). Specific density, also termed functional specific gravity, is the dominant factor to affect particle outflow from the rumen. Functional specific gravity value (FSG) is the ratio of the density of a substance to the density of a reference substance like water (Evans et al., 1973). Khan et al. (2021) demonstrated that specific density did not correlate with bulk density for high-density feed pellets when analyzing extruded pellets behavior in rumen fluid. It has been shown that rumination started at the time of maximum concentration of particles of low density and minimum concentration of particles of high density (Evans et al., 1973). In this study, a volumetric displacement method determines the specific density of the pellet by using glass beads with a diameter of 0.1 mm as a displacement medium.

Sinking velocity

Sinking velocity is also normally used in the fish industry. It is measured as the time of pellets passing through a certain distance by dropping pellets one by one above the water surface. A stopwatch is used to record the time over two fixed points. However, it also can be used to determine the pellet of sinking speed in the rumen by replacing water with rumen fluid. Single pellets are selected randomly for measurement, and sinking velocity is recorded as mm s-1 (SØRensen, 2012).

Fluid stability

Fluid stability is determined by modifying the water stability test used in the fish industry. Water stability of feed is essential for slow eating quantic animals to mimic the degradation pattern of feed in the digestive tract since the pellet has to be soaked in water for hours (SØRensen, 2012). Similarly, fluid stability replaced water with rumen fluid mimic the process of the feed in the rumen, which also can be used to determine the degradation pattern of feed in the rumen. Pellet samples are added into circular wire netting baskets with 2.5 mm mesh size. The baskets with samples are placed in the beaker and rumen fluid is added. The samples are incubated at 39°C and subjected to continuous shaking.

After finish incubation, the baskets are removed from rumen fluid and clear the surface of the outside. Then the baskets were weighed and dried at 105°C for 18h. After drying, the basket was weighed again to determine the residual. The fluid stability was calculated as the difference in DM weight after incubation divided by before incubation.

Durability

Durability is another physical quality parameter of feed pellets, measured as the amount of fines returned from a batch of feed pellets under standard conditions (Thomas & van der Poel, 1996). Testing of pellet durability mimics the force on pellet taking place during filling of bins, during transportation from the feed producer to the farm, and during distribution in the feeding system at the farm. Pellets with high durability produce less fines (SØRensen, 2012).

Holmen durability tester is a common method to test durability. In this method, a 100 g pellet sample is transported around a closed circuit using air. After a standardized time of 30 to 120 s, the pellet is sieved in a sieve with a sieve opening of 80% of the pellet diameter. Finally, the durability is expressed as PDI calculated as the mass of the pellets retained on the sieve divided by the total mass of pellets (SØRensen, 2012).

Degradation

Ruminal degradation is usually determined *in vitro*, *in sacco,* or *in vivo*. *In vivo* is a time-consuming and expensive method to measure digestibility. The standard method is measuring the total feed input and output in faeces and urine. *In vitro* is a simple, substrate-specific, and labour-efficient method that provides alternatives to animal-dependent experiments used *in situ* or *in vivo* methods. The feed sample is incubated with rumen fluid or pepsin and acid. However, intrinsic factors are not measured since many *in vitro* systems fail to include adequate buffers, reagents, or equipment to guarantee the pH, anaerobiosis, redox potential, microbial numbers, and essential nutrition provided to microbes. The *in sacco* method is another way to measure the degradation of pellets. The measurement is by putting feed samples into nylon bags and making them incubate in the rumen. After incubation, the bags are washed and dried, and the rest material is analysed. Compared with *in vitro*, *In sacco* method is available to determine the combined effects of the intrinsic properties of both feed and the animal (Dijkstra et al., 2005).

3. Material and methods

3.1 Feed ingredients and feed processing

The pellets were produced at the Center of Feed Technology (ForTek) at the Norwegian University of Life Science, Ås, Norway. Three diets are based on the same recipe of barley and soybean meal (SBM), and their ratio in the diet is 70:30, respectively. Barley and SBM were ground separately in the hammer mill (HM 21.115, Munch-Wuppertal, Germany) with 2 mm screen sizes and then mixed in a twin shaft paddle mixer (Forberg AS, Larvik, Norway) for 120 s. Three diets were produced with three treatments in which diet 1 and 2 were produced in the extrusion process, and diet 3 was produced in expanding process. Three treatment process parameters are shown in table 2. Diets 3 as a control diet (Con) was produced with the expanding process followed by pelleting with a conventional pellet press. The feed remained in the conditioner for only seconds, where it was heated to 84 ºC. The temperature reached 111 ºC in the expander. The pressure in the expander was 9.68 bar. Diet 1 (LD) and diet 2 (HD) were produced in the extrusion process with similar total water percentages but different screw speeds of 275 and 200 rpm, respectively. Diet 1 emerged from the extruder at 90-127 ºC, whereas diet 2 was at 88-110 ºC. Each diet has three replications.

Table 2 Extrusion and expanding process data for 70:30 barley to soybean meal.

Process parameter	Extrusion LD			Extrusion HD			Expanding Con		
sample	1	2	3	1	2	3	1	2	3
Die size (mm)	3			3					
Cond. Temp. (°C)	90			90			84.7		
Cond. steam(kg/h)	10.8			12					
Cond. water (kg/h)	14.8			19.5					
Total water %	25.6			27.2					
Barrel 1 (°C)	90.6			88.5					
Barrel 2 (°C)	120.7			110.9					
Barrel 3 (°C)	127.1			105.9					
Barrel 4 (°C)	126.4			102.3					
Barrel 5 (°C)	124.4			84.6					
Pressure, barrel 4 (bar)	1.41			0.01					
Expander Temp. (°C)							111		
Pressure (cone) (bar)							9.67		
Die temperature (°C)	119.2			85.4					
Screw speed (rpm)	275			200					
Torque (Nm)	316.4			390.1					

3.2 Measuring physical properties of pellet

Hardness

Hardness was determined by a KAHL Pellet Hardness tester (A. KAHL GmbH, Reinbek, Germany). The tester was equipped with a 2.5mm spring with a 0-25 kg compression pressure range. The test was conducted by setting the indicator on 0 marks and then locate a pellet between the anvil and piston top and tighten the screw until the pellet burst. In total, 15 pellets are tested randomly for each diet.

Durability

Pellet durability was determined in Holmen (NHP200 Automated Feed Pellet Durability Tester, TEKPRO, Britain). In this method, 100 g samples were weighted and placed into a chamber. The whole pellets that remained on a 3 mm screen were weighed on an electronic scale. The pellet durability index (PDI) was calculated as the percentage of pellets remaining after tumbling. Each of the samples does three replications.

Bulk density

Bulk density was tested by a 1 L plastic cylinder. Pellets were poured into cylinder mild until a pile of feed had formed on top. A scraper was used to remove the excess pellet over the edge of the cylinder. Then the cylinder with pellet was weighed and recorded value. Each measurement was carried out in triplicate. And the bulk density was calculated as ρ (g L^{-1}) = mass of sample / unite volume of sample.

Specific density

The change of specific density of pellet was determined in a trapped density analyser (AUTOTAP, Quantachrome Instruments, Boynton Beach, Florida, USA). Pellet volume was determined by the volumetric displacement method. This study test specific density for 0, 10, 20, 30 min incubation in rumen fluid. Adjust the tapping rate of the trapped density analyser to 200 taps per minute. The volume of a 7.5 ml glass beads with 0.5 µm diameter is placed in a 10 ml cylinder and detected by trapped density analyser, recorded as initial volume. For 0 min incubation, seven pellets were selected randomly, weighed, and dropped into a 10 ml cylinder one by one. Glass beads recorded as initial volume are used to fill these seven pellets. Then the volume with seven pellets was detected in a trapped density analyser, recorded as the final volume. The pellet volume is that the final volume minus the initial volume. The specific density of pellets (g/ml) is that the seven pellet weights divide by pellet volume. For testing pellet, specific density for 10, 20, 30 min incubation, the pellets were incubated in rumen fluid at 39°C in the oven. After incubation, the rumen fluid is removed from the pellet surface, and their detection method of specific density is similar to dry pellet in 0 min incubation.

Sinking velocity

The sinking velocity of the pellet was tested by a 250 ml cylinder (310 mm high and 35 inner diameters) filled with strained rumen fluid. This test was performed in the incubator at 39 °C. 30 pellets of each sample were chosen randomly and dropped to cylinder one by one. Record the time of pellet passing a distance of 220 mm in a transparent glass cylinder. A total of 270 pellets were analysed.

Fluid stability

Fluid stability was determined in the Ankom Daisy incubator (Ankom Technology Corp, NY, USA). Rumen fluid was collected from 3 fistulated Norwegian Red cows and was strained through gauze with the size of 200 µm and then divided into three jars. A 5 g sample of each diet was added in circular wire netting baskets (inner diameter: 55-58 mm; mesh size: 0.7 mm). Each diet had three samples. Moreover, every sample had three replications. Totally nine samples of each diet were incubated at 39 °C for 15, 30, 60, 90 min respectively. After incubation, the liquid and particles attached to the outside surface of the basket are removed by paper. Then the baskets were weighed and dried at 105°C for 18h. After drying, the baskets were weighed again to determine the residual.

3.3 Rumen degradation of dry matter

Pellet degradation was determined by *in sacco* method (Åkerlind et al., 2011). Each diet's sample was divided into two samples where one group sample was ground with a 2.5 mm ring mill. Samples (2 g) were placed into numbered nylon bags with a pore size of 36µm. The seams of the bags were sealed with a rubber band to prevent samples from escaping during incubation. The sample bags were attached with plastic string which keeps sample sediment in the rumen liquid. Bags were incubated in rumen fistulated cows for 0, 2, 4, 8, 16, 24, 48 h. After incubation, bags were rinsed with cold water. After then, washed bags were dried at 45°C for 48 h. Dry bags were placed at room temperature for 24 h and then weighed the residue.

3.4 Statistical analysis

Treatment of durability, hardness, bulk density, sinking velocity, and specific density were statistically analysed with GLM procedure of SAS where treatment was the main effect. Treatments of fluid stability were statistically analysed with the MIXED procedure of SAS. The *in sacco* data was analysed with the MIXED procedure of SAS using a model with treatment and cow as fixed effects. The following predefined contrasts were tested: high density (HD and Con) pellets versus low density (LD) pellets,

expressed as LD vs. HD; extruded pellets versus expanded pellets, expressed as Extruded vs. Con. The results are reported as least square (LS) means with a standard error of the mean (SEM). Significance was claimed when $P \leq 0.05$ and tendencies were considered as $0.05 < P \leq 0.10$.

4. Results

4.1 Physical property analyses of three treatment concentrate

As shown in table 3, durability in the three treatments is significantly different ($p<0.001$) in which durability of Con group (92%) is significantly lower than extrusion group, including LD (95.8%) and HD (99%) (Table 3). Hardness for these three treatments is also significantly different ($p<0.001$), and it was observed that the LD diet has a very low value (49.64 N) and the HD diet has the highest value (112.01 N). Concerning sinking velocity (SV), it is also significantly different between the three diets, where LD group is floating and the SV value is 0.00, the control group has a higher SV (20.64 mm/sec) than the HD group (9.79 mm/sec). There is no specific density difference in the dry state (SPD) between the HD and the control group. In contrast, these two groups are significantly different from the LD group ($p<0.001$) and the LD group has the lowest SPD value (0.70 g/ml). It showed a significant difference in LD and HD group related with specific density in dry and incubation state ($p<0.001$), and their value are 0.84 g/ml and 1.08 g/ml, respectively. Bulk density (BD) in the three treatments is significantly different ($p<0.001$) and the BD value of LD (413.2 g/L) and HD group (624.7 g/L) are lower than the control group (650 g/L).

Table 3 Physical properties of pellet.

Item 2	Treatments1			SEM3	P-value
	LD	HD	Con		
PDI (%)	95.8b	99.0a	92.0c	0.34	<0.001
Hardness (N)	49.64c	112.01a	90.50b	2.01	<0.001
SV (mm/sec)	0.00c	9.79b	20.64a	0.13	<0.001
SD (g/mL)	0.70b	1.03a	1.07a	0.02	<0.001
SPDW (g/ml)	0.84b	1.08a			<0.001
FSI (%)	87.8a	92.2a	34.2b	2.34	<0.001
BD (g/L)	412.3c	624.7b	650.0a	2.53	<0.001

[1] LD= Low-density extruded pellet, HD= High-density extruded pellet, Con = Expanded pellet
[2] PDI= Durability, SV= Sinking velocity, FSI= fluid stability, SPD= Specific density in dry state, SPDW = Specific density tested in dry and incubation in rumen fluid, BD= Bulk density
[3] Standard error of means

4.2 Fluid stability of three treatment concentrate varies with time.

As shown in figure 6, fluid stability (FSL) of the control group decreases largely from 60.33 % in 15min incubation to 13.08% in 90 min incubation. Compared with the control group, there is no significant decrease of FSL in extruded concentrate, including HD and LD, and their FSL are 96.75 % and 95.21 %, 97.26 % and 96.90 % respectively in 15 min and 30 min incubation. Whereas HD group has higher FSL than LD in 60 min and 90 min incubation, their values are 90.27% and 84.65%, 86.82% and 72.49% respectively in HD and LD.

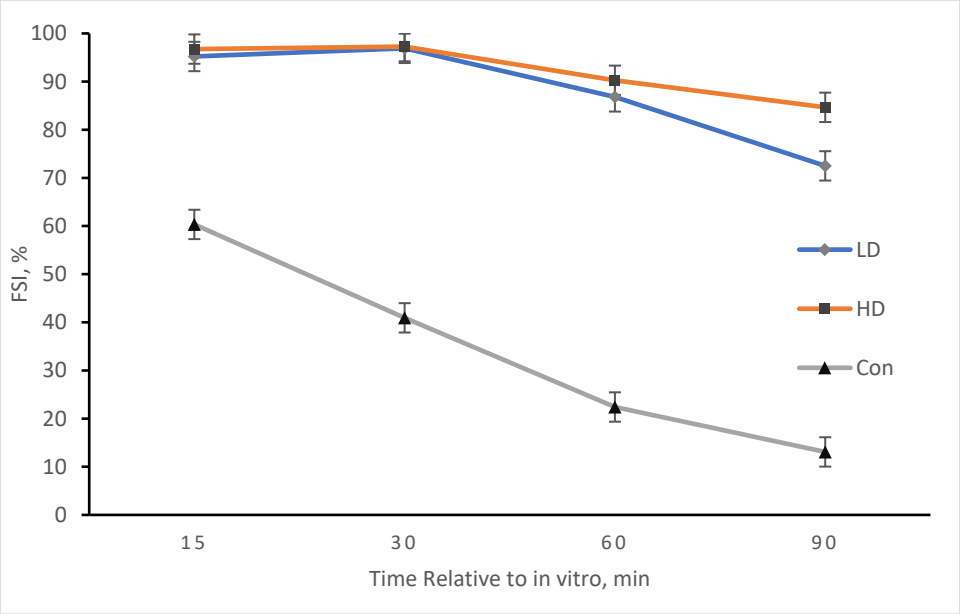

Figure 6. Fluid stability of LD, HD and Con vary with time (FSl = fluid stability).

4.3 Result of specific density change vary with time for three treatments

As figure 7 shown, the specific density of the three treatments increases with time. The Control group has the highest specific density compared with LD and HD and is increased from 1.07 g/ml to 1.14 g/ml during 10 min. The specific density of the HD group is increased slowly from 1.03 g/ml in the dry state, 1.07 g/ml in 10 min incubation, 1.08 g/ml in 20 min incubation to 1.13 g/ml in 30 min incubation. For the LD group, specific density during 30 min incubation is still lower than 1 g/ml, although it constantly increases from 0.7 g/ml to 0.93 g/ml from 0 min to 30 min incubation.

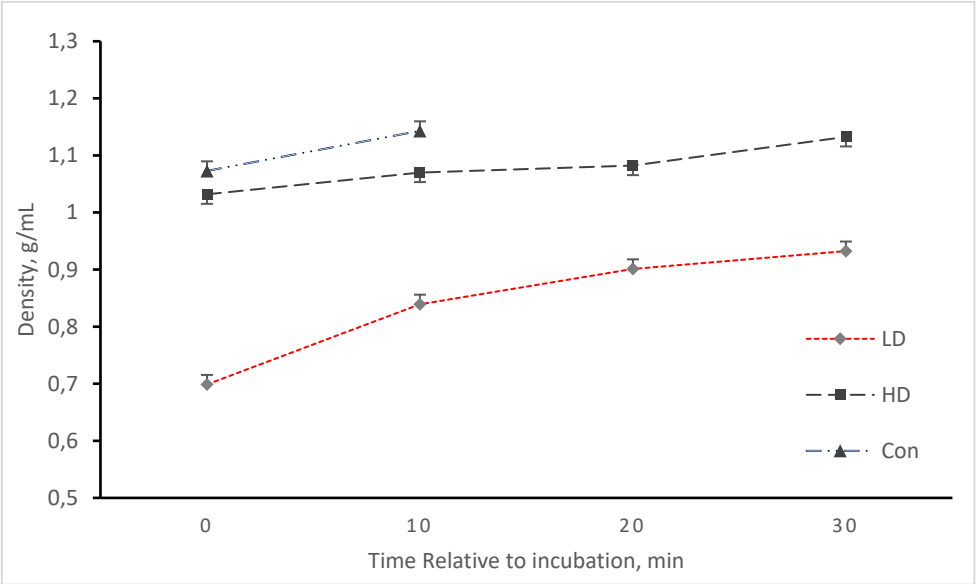

Figure 7. Specific density tests of LD, HD and Con vary with time

4.4 *In sacco* measurement

The result of *In sacco* degradation of dry matter for pelleted and ground concentrate of three treatments is shown in the table 4 and 5, respectively. There are similar results about soluble fraction (A), potentially degradable fraction (B), rate of degradation of B (C) and Total degradation of dry matter (D). Compared with the Con group, A in LD and HD is significantly decreased ($p<0.001$). Whereas B and C of LD and HD groups are significantly higher than the Con group ($p<0.001$). D in the extrusion process is significantly decreased as compared with expanding press process ($p=0.001$). Besides, some differences occurred in Effective dry matter degradability (ESD). For pelleted concentrate, ESD of HD is commonly the lowest among the three treatments ether in the rate of passage of 0.05 h-1 (ESD5), 0.06 h-1(ESD6), 0.07 h-1 (ESD7), or 0.08 h-1(ESD8). Whereas it is the highest value in ground concentrate.

In this study, intact pellets were used during *in sacco* to observe the effects of the physical properties of pellets on rumen degradation. Use ground samples as a standard procedure in which a high ESD is observed in extruded HD concentrate.

Table 4 In sacco degradation of dry matter for pelleted concentrate

	Treatments[1]			SEM[3]	P	-value	
Item[2]	LD	HD	Con			Extru vs con	LDen vs HDen
ED_5 kg/d	73.56^{ab}	72.07^{b}	74.73^{a}	0.32	0.011	0.008	0.710
ED_6 kg/d	71.68^{b}	69.60^{c}	72.41^{a}	0.33	0.009	0.012	0.171
ED_7 kg/d	69.91^{ab}	67.31^{b}	70.31^{a}	0.33	0.006	0.014	0.054
ED_8 kg/d	68.24^{a}	65.19^{b}	68.41^{a}	0.33	0.004	0.014	0.023
A	7.50^{b}	7.34^{b}	27.35^{a}	0.63	<0.001	<0.001	<0.001
B	77.42^{a}	80.94^{a}	64.28^{b}	0.78	<0.001	<0.001	0.007
C	29.97^{a}	20.72^{b}	14.92^{c}	0.80	<0.001	<0.001	<0.001
D	84.92^{c}	88.29^{b}	91.62^{a}	0.52	0.002	0.001	0.001

[1] LD= Low-density extruded pellet, HD= High-density extruded pellet, Con = Expanded pellet, Extru vs con = extruded pellets vs expanded pellets, LDen vs HDen = Low density pellets (LD) vs High density pellets (HD + Con)

[2] ED: Effective dry matter degradability calculated using a fractional rate of passage of 0.05 h^{-1} (ED_5), 0.06 h^{-1}(ED_6), 0.07 h^{-1} (ED_7) or 0.08 h^{-1}(ED_8)

A= Soluble fraction (%), B= Potentially degradable fraction (%), C=rate of degradation (%), D= total degradation (%)

[3] Standard error of Means

Table 5 In sacco degradation of dry matter for ground concentrate

	Treatments [1]			SEM[3]	P		-value
Item[2]	LD	HD	Con			Extru vs Con	LDen vs HDen
ED_5 kg/d	75.95^{b}	77.47^{a}	77.45^{a}	0.14	0.003	0.013	0.001
ED_6 kg/d	74.52^{c}	76.00^{a}	75.38^{b}	0.11	0.002	0.421	0.001
ED_7 kg/d	73.16^{b}	74.59^{a}	73.55^{b}	0.09	0.001	0.043	0.001
ED_8 kg/d	71.90^{b}	73.26^{a}	71.91^{b}	0.08	<0.001	0.002	0.002
A	17.92^{c}	18.72^{b}	42.71^{a}	0.14	<0.001	<0.001	<0.001
B	66.40^{a}	67.42^{a}	50.86^{b}	0.30	<0.001	<0.001	<0.001
C	34.74^{a}	33.91^{a}	10.79^{b}	0.89	<.0001	<0.001	<0.001
D	84.32^{b}	86.14^{b}	93.57^{a}	0.41	<.0001	<0.001	<0.001

[1] LD= Low-density extruded pellet, HD= High-density extruded pellet, Con = Expanded pellet Extru vs con = extruded pellets vs expanded pellets, LDen vs HDen = Low density pellets (LD) vs High density pellets (HD + Con)

[2] ED: Effective dry matter degradability calculated using a fractional rate of passage of 0.05 h^{-1} (ED_5), 0.06 h^{-1}(ED_6), 0.07 h^{-1} (ED_7) or 0.08 h^{-1}(ED_8)

A= Soluble fraction (%), B= Potentially degradable fraction (%), C=rate of degradation (%), D= total degradation (%)

[3] Standard error of Means

In sacco rumen, degradation of pelleted and ground pellet dry matter (DM) comparison is shown in figure 8. It can be observed that pelleted and ground concentrate has a similar trend where degradation increase with incubation time. What the difference is that degradation of ground concentrate is higher than pelleted concentrate. Besides, the degradation of pelleted HD (P-HD) is lower than in other groups. However, eventually, these concentrate groups almost reach the same degradation value at 48 h.

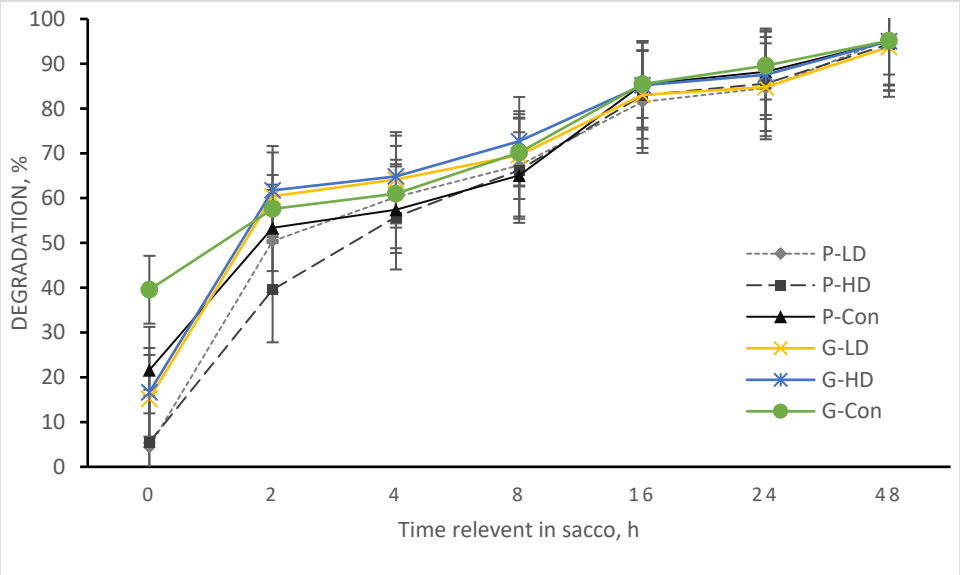

Figure 8. Degradation of pelleted and ground concentrate vary with time (P-LD = pelleted LD concentrate, P-HD = Pelleted HD concentrate, P-Con = pelleted Con concentrate, G-LD = Ground LD concentrate, G-HD = Ground HD concentrate, G-Con = Ground Con concentrate)

5. Discussion

5.1 Physical quality

Physical quality including durability, hardness, bulk density, specific density, fluid stability, and sinking speed was measured. Distinct differences in the physical quality of pellet between treatments were observed. According to Kaliyan and Morey (2009), the strength and durability of densified products like pellets depend on chemical composition, moisture content and particles size of the feed, the pre-conditioning process and use of binders, and the processing equipment.

Pellets that had been extruded exhibited higher PDI value than expanded pellets (Table 3). A possible reason is that there is added higher temperature and more moisture level to the extrusion process than expanding process, and components in this process suffer from long cooking time. Although the chemical analysis in this research was not measured, it is speculated that HD and LD have high gelatinized starch than Con, which improves their physical properties, including durability. This is also in accordance with Loar et al. (2014), where durability is lower in expanded pellets and higher in extruded pellets.

Another possible reason is the effect of denaturing protein as both gelatinisation of starch and denaturing of protein affects pellet quality positively (Wood, 1987). As previously reported research (Kaliyan & Vance Morey, 2009) showed that proteins from cereal grains with dough-forming capabilities such as wheat, barley and soybean meal under combined effects of thermal and mechanical effects result in protein denaturation, which has a positive effect on pellet durability. In contrast, Kaliyan and Vance Morey (2009) found that pellet durability was reduced with the use of protein derived from corn. That means protein denaturation cannot be used as a principle to explain all the situations related to pellet physical properties. It should be discussed according to the different protein sources.

In addition to starch and protein, It is reasonable to speculate that fibre also acts as a binder since Kaliyan and Vance Morey (2009) reported that durability increase from 93.8 to 97.4% if feed increase fibre content from 4.4 to 14.0%. There are water-soluble fibre and water-insoluble fibre. Water-soluble fibre acted as a binder was reported in Kaliyan and Vance Morey (2009), which may be explained by its viscous characteristic as meeting with water and high temperature. Thereby the water-soluble fibre is adhered to the surfaces of solid particles to generate strong bonds, resulting in high durability, hardness, and fluid stability in the extruded pellet of HD and LD (Table.3). Although water-insoluble fibre negatively affects pellet durability, it is speculated that this negative effect is reduced by the extruded process (Table.2). It is supported by Razzaghi et al. (2016) that exposure of

insoluble fibre molecules to shear by extrusion result in breakage of chemical bonds, thus creating smaller and soluble particles. It was also reported that cooking extrusion increased the soluble fibre content in barley flour indicating a shift from insoluble dietary fibre to soluble dietary fibre (Vasanthan et al., 2002). So It is reasonable to speculate that the water-soluble fibre plays a positive effect on pellet durability, the negative effect of water-insoluble fibre is reduced by extruded process.

Compared to HD and LD diets, it found PDI reduction with increased temperature (Table.3). The lower PDI in the LD diet may be explained by a generally more expanded and porous structure of this diet, as confirmed by the markedly reduced bulk density (Table 3). An inverse relationship between expansion on one side, and bulk density, hardness and durability on the other side is shown in Lundblad et al. (2011). It is a hypothesis that high durability may reduce fine particles production during rumination and thus keep pellets intact as much as possible. More nutrients are the potential to escape from the rumen. However, information about the effects of pellet durability on the performance of cattle is limited. More research is needed to investigate.

A significantly high value for the HD group compared to the LD group with respect to hardness corresponds with (Khan, 2021). It is partly explained by the methods used to test hardness, and it has been shown that the hardness of HD is markedly higher than LD if measured with a knife knob but similar when measured with a flat knob (Khan, 2021). It also can be predicted that high hardness will intensify the chewing process, which may reduce the ingestion of pellets due to the high breaking force in HD. In animals, high hardness may also increase saliva production, as shown in a previous study (Bochnia et al., 2019). The palatability is potentially decreased in HD due to its high hardness since it is difficult to chew these high hardness pellets by cattle. Whereas this can be overcome by using a small size of pellets as shown in Khan (2021) that cattle fed with a die size of 3-4 mm improved feed intake as compared with 6 mm.

According to a previous study, sinking velocity (SV) is strongly correlated with bulk density and specific density (Khan, 2021). He found that pellets exhibit floating characteristics at a BD <430 g/L or specific density (SD) < 0.78 g/mL, and exhibit sinking characteristics at a BD range from 600-740 g/L or SD at 0.97-1.12 g/mL (Khan, 2021). This corresponds well with my observations where the LD group was floating, and the HD groups were sinking.

The density of feed particles influences the probability of rumen escape (Dufreneix et al., 2019). Low density particles will float in the rumen, whereas high-density particles will sediment. Thus, SD where the probability of rumen escape is at the highest point was suggested to be above 1.05 g/ml by Khan (2021). In my study, Con expanded pellets were the only feed exceeding this level, whereas HD extruded pellets were close to that level (Table 3). Feed particles change their specific density if they

are in rumen fluid. The specific density of LD increase during 30 min but was still lower than 1.00 g/ml. Whereas HD has increased from 1.03 to 1.13 g/ml. Hence it can be expected that HD will have a low retention time in rumen since Seyama et al. (2017) and Dufreneix et al. (2019) indicated that high-density particles (from 1.17 to 1.42 g/ml) have a higher probability for rumen escape than low density particles (less than 1.00 g/ml). The probability of rumen escape is a balance between passage and degradation. The probability of passage increases with increased density. However, to exhibit the probability of rumen escape, feed pellets cannot disintegrate in the rumen, losing their SD properties. In that respect is stability in rumen fluid crucial. Extruded LD and HD diets pellets have significantly higher rumen stability than expanded pellets as HD expanded pellets were fully disintegrated after 10 minutes (Figure 7), which corresponds with Khan (2021). Measuring stability of various extruded pellets in water, Razzaghi et al. (2016) also found considerable variation in stability.

5.2 Effect of physical quality on rumen degradation of dry matter

To simulate the effect of processing on rumen degradation of dry matter, the *in sacco* experiment was conducted. The standard procedure for *in sacco* is to grind samples using a 1.5 mm screen (Åkerlind et al., 2011). However, grinding the pelleted samples to a powder would make them lose their physical properties, probably influencing their degradation *in sacco*. Based on the *in sacco* degradation, it indicates that all pellets extruder processed exhibited low soluble fraction (A) compared to expander processed pellets. Previous research also observed this by comparing the extrusion and the expanding process (Khan, 2021). The effect was present measured both on intact and ground pellets, but the soluble fraction increased by grinding. Increased soluble fraction could be related to reduced particle size and thereby increased particle loss due to grinding (Åkerlind et al., 2011). As total degradation was not influenced, reduced A fraction increased the potentially degradable fraction (B) accordingly. As a result, the rate of degradation of C was not expected to change dramatically. However, for the HD extruded pellets, a considerable increase in the rate of degradation by grinding was observed. HD extruded pellets were the pellets showing the highest fluid stability. This indicates that grinding mask the influence of the physical property of the HD extruded pellets. A possible explanation is that concentrate pellets produced with the extrusion process have a high level of gelatinization, gluing components together and thus resulting in high fluid stability. The water can not enter the inner part of pellets and thus prevent inner particles from leaching out. This particle gluing was probably greater for high-density extruded pellets than for low density extruded pellets, as indicated by a relatively high FSI for high-density extruded pellets. Extrusion can induce chemical reactions between starch molecules within the granules, increasing starch crystallinity and thus decrease ruminal degradation (Razzaghi et al., 2016).

Moreover, Maillard reactions that occur in heat treatment may protect starch from degradation. Starch is the main component in the pellet. Thus it is reasonable to speculate that Maillard reactions protect extruded pellet (DM) from degradation. However, as ED was lowest measured on intact pellets and highest measured on ground pellets, the influence of grinding prior to *in sacco* exceeded the potential effects of pellet quality on *in sacco* degradation of dry matter.

5.3. Implications of processing on the nutritional value of feed pellets

Pellet produced in the different processes affects its dynamic change in the rumen. However, this has not been implemented in the current feeding system like NorFor (Volden, 2011). So this study can be utilized for the predictions of extends of rumen degradation and escape. Distinct differences of rumen degradation between treatments were observed. Feed type, pellet quality, feeding level, and feed process may modify the rate of passage and digestion. Pellet with high density and rumen stability is speculated to have lower rumen degradability and higher rumen passage rate. This statement is confirmed by the evidence that extruded pellets (HD) reduced DM degradation (Figure.8) and ED (Table.4) than LD and Con. It is also supported by Arieli et al. (1995) that ruminal escape of DM increased by 8% and 5%, in extruded and expanded grains, respectively. Although the chemical analysis of starch and protein was not measured, expanded pellets have the highest soluble fraction of DM (Table.4). It is speculated that it also has the highest soluble fraction of starch and protein as Niu (2018) reported. The soluble starch and protein were lost through the bag pores during *in sacco* and a possible reason for this highest loss in expanded pellets is due to their low FSI. Compared with expanded pellets, the extent of gelatinized starch is higher in extrusion, which glue particles tightly and result in higher FSI. This gluing property is higher in HD than LD, as indicated by a relatively high FSI in HD (Table 3). Thereby it is reasonable to speculate that the effective degradation of starch (EDS) is lower in HD than LD. This speculation is in accordance with Khan (2021) reported that pellets with high fluid stability shown lower EDS than pellets with low fluid stability. The reduction of effective protein degradation (EDP) in the extruded pellet is speculated since many studies have confirmed that protein degradation is reduced in the rumen. For instance, Mendowski et al. (2019) shown that faba bean protein in the extruded process was less degradable in the rumen and this degradation was decreased with increased temperature. Whereas the protein produced at high temperature (higher than 160 °C) leads to overprotection of feed proteins and thus reduces amino acid availability in the intestine (Mendowski et al., 2019). Previous research also reported that oats need a higher temperature than barley, wheat and wheat bran to achieve a lower rate of protein degradation (Ljøkjel et al., 2003b). The variation in ESD may partly be related to the construction of the protein–starch matrix that differs among the feeds. Protein and starch form a more rigid matrix in barley but appear

as a loose arrangement in oats. This loose structure is attacked easily by microorganisms or enzymes so that the EDS and EDP are higher (Ljøkjel et al., 2003b). It also can speculate that rumen degradation of lysine was reduced in the extrusion process than in the expanded process. A part of the reason to explain it is that some of the cross-linking reactions and formation of new amino acids like lysinoalanine reduced protein degraded in rumen need longer reaction time (Ljøkjel et al., 2003a). Millard reactions occurred at high temperature is another reason to protect lysine from rumen degradation (Ljøkjel et al., 2003a). The reactions between lysine and sugar increased with cooking time and temperature protects the protein from microbial degradation in the rumen (Kostyukovsky & Marounek, 1995). Contrary to EDS and EDP, the effective rumen degradability of neutral detergent fibre (NDF) is speculated slightly affected by the expanded process but tends to increase in the extrusion process. This is supported by Razzaghi et al. (2016) that extrusion reduces the molecular weight of pectin and hemicellulose molecules, resulting in increased water solubility of fibre and increased the effective NDF degradability accordingly. The reduced NDF is also observed in other research like Camire et al. (1990) and Prestløkken and Harstad (2001). Both heat treatment and shearing force result in breakage of water-insoluble fibre thus creating smaller and soluble particles, but steam addition observed did not increase the effective NDF degradability in the fibre mixtures and the reason is unclear (Razzaghi et al., 2016).

6. Conclusion

It is concluded that pellet physical quality can be modified by feed processing. Pellets with high density and fluid stability were achieved if extruded at low temperatures. It is difficult to conclude on rumen starch, and protein degradation as only dry matter degradation was measured in the present study. Combined with previous research, the probability is that the extruder process reduces the soluble fraction, thereby reducing effective degradation accordingly. High density has the lowest soluble fraction and the effective degradation due to high water stability. It seems that the high density and fluid stability pellets could increase the passage rate and decrease the degradation rate increasing rumen escape of pellets. More research related to changes like rumen starch and protein degradation need to be finished to verify this.

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