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Effects of extruded pellets on physical feed quality and digestion behavior in dairy cows

Effekt av ekstrudert pellets på fysisk fôrkvalitet og fordøyelsesegenskaper hos melkekyr

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Declaration

I, Puchun Niu, declare that this thesis is a result of my research investigations and findings. 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 award of any type of academic degree.

Signature…….

Date……16.12.2018…….

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Puchun Niu

Summary

This thesis consists of a literature study and a presentation of an experimental work. The literature review contains knowledge of raw ingredients, rumen digestion of starch and protein and feed processing techniques. The purpose of the experiment was to examine the effects of extruder processing on physical quality of concentrate feeds, and on rate of rumen digestion and rumen outflow of starch and protein.

Nine concentrates in the pilot production with barley and SBM at the ratio of 65: 35, ground on either 2 or 4 mm hammer mill screen, were extruded using die size either 3 or 5 mm. The nine concentrates were divided into three groups (1) using 2 mm screen and 3 mm die, (2) using 2 mm screen and 5 mm die, and (3) using 4 mm screen and 5 mm die. Three concentrates in each group were extruded (1) with steam injection into 4^{th} section of the extruder barrel, (2) with water cooling of the last section of the extruder barrel, and (3) as such (with neither steam injection nor water cooling), respectively. In the second step, concentrates from first group were selected based on physical property test and were reproduced as experimental concentrates. The concentrate used as control was expanded. All four experimental concentrates had a ratio of barley and SBM at 70: 30.

The animal experiment was designed as a 4*4 Latin square, with four experimental diets consisting of the four concentrates (daily ration of 10 kg/cow) together with grass silage (*ad libitum*); four multiparous lactating Norwegian Red cows, equipped with rumen and duodenal cannulas; and four periods, each lasting for 21 days. Ruminal degradability of DM, starch and protein was measured *in sacco* (0, 4, 8, 24 and 48 hours). Rumen fermentation patterns, rumen digestibility and total tract digestibility of starch and protein were measured *in vivo,* through collecting samples from rumen fluid, duodenum, and feces and urine, respectively.

Grinding on 4 mm screen, concentrates extruded with steam injection and as such in the pilot production showed higher bulk density (570-600 g/L) compared to concentrates (439-513 g/L) ground on 2 mm screen. Concentrates extruded with water cooling showed high bulk density (588-611 g/L) regardless of screen size. Concentrates with bulk density from 570 to 611 g/L were rapidly sinking in rumen liquor. Concentrates ground on 4 mm screen had lower water stability index (WSI) than concentrates ground on 2 mm screen. From the second production, the extruder treated concentrates showed higher WSI than the expanded concentrate (P<0.05). The low, medium and high bulk densities (410, 545, 610 g/L) corresponded to floating, slow sinking and

fast sinking extruded concentrates. Extruder treatment considerably reduced effective DM degradability (EDMD) and effective starch degradability (ESD) *in sacco* compared to the expander treatment. The reduction on ESD was most pronounced for the concentrate with highest WSI. EPD for the four concentrates were significantly different from each other ($P<0.05$) and was minimized by extruder treatment with steam injection. In agreement with ESD measured *in sacco*, rumen starch digestibility *in vivo* tended to decrease ($P=0.08$) for concentrate extruded with water cooling, which also had highest WSI, compared to expanded concentrate. Concentrate extruded with steam injection showed nominally lowest ruminal protein degradability compared to the other concentrates, consistent with EPD measured *in sacco*. Processing method of concentrate neither altered total tract digestibility of DM, starch nor of crude protein.

Extruder treatment can be used to alter degradation rate of DM and single nutrients in concentrates to dairy cows. It is possible to alter rumen digestibility without altering total tract digestibility, indicating an increase of nutrient digestion in the small intestine.

Key words: Extruder processing, physical quality, rumen degradation, *in sacco*, rumen digestibility, *in vivo.*

Sammendrag

Denne oppgaven består av en litteraturstudie og en presentasjon av et eksperimentelt arbeid. Litteraturvurderingen inneholder kunnskap om råvarer, fordøyelse av stivelse og protein i vom og fôrbehandlingsteknikker. Formålet med eksperimentet var å undersøke effekten av ekstruderprosessering på fysisk egenskap av kraftfôr, og på hastighet av fordøyelse og utstrømming av stivelse og protein fra vom.

I en pilotproduksjon ble bygg og soyamel (SBM) blandet i forhold 65:35 malt på hammermølle med enten 2 eller 4 mm sold og ekstrudert ved en dysestørrelse på enten 3 eller 5 mm. Det ble produsert ni prøver delt inn i tre grupper ved hjelp av (1) 2 mm sold og 3 mm dyse, (2) 2 mm sold og 5 mm dyse, og (3) 4 mm sold og 5 mm dyse. Tre prøver i hver gruppe ble ekstrudert med (1) dampinjeksjon i fjerde seksjon av ekstruderen, med (2) vannkjøling av den siste (femte) seksjonen av ekstruderen, og (3) som sådan (med hverken dampinjeksjon eller vannkjøling). Ut fra de fysiske egenskapstestene i pilotproduksjonen ble de tre prøvene fra første gruppe valgt ut og reprodusert som eksperimentelle dietter. En ekspandert prøve ble anvendt som kontrolldiett. Alle fire eksperimentelle dietter hadde et forhold mellom bygg og SBM på 70:30.

Dyreforsøket ble gjennomført som et 4 * 4 Latinsk kvadrat med de fire eksperimentelle diettene som forsøkskraftfôr. Den daglige rasjonen var 10 kg kraftfôr pr. ku og grassurfôr gitt *ad libitum*. Fire voksne lakterende kyr av rasen Norsk rødt fe med kanyle i vom og duodenum ble benyttet. Det var fire forsøksperioder, hver på 21 dager. Nedbrytbarhet av tørrstoff, stivelse og protein i vom ble målt *in sacco* med inkubasjonstider på 0, 4, 8, 24 og 48 timer i vom. Gjæringsmønster i vom, fordøyelighet i vom og fordøyelighet totalt ble målt *in vivo* ved å samle prøver fra henholdsvis vomvæske, tolvfingertarm og avføring og urin.

I pilotproduksjonen viste prøver malt på 4 mm sold og ekstrudert med dampinjeksjon og som sådan, høyere egenvekt (570-600 g/L) sammenlignet med prøver malt på 2 mm skjerm (439-513 g/L). Prøver ekstrudert med vannkjøling viste høy egenvekt (588-611 g/L) uavhengig av soldstørrelse. Prøver med egenvekt fra 570 til 611 g/L var raskt synkende i vomvæske. Prøver malt på 4 mm sold hadde lavere vannstabilitetsindeks (WSI) enn prøver malt på 2 mm sold. Fra den andre produksjonen viste de ekstruderte diettene høyere WSI enn den ekspanderte dietten ($P<0.05$). Lav, middels og høy egenvekt (410, 545, 610 g/L) korresponderte henholdsvis med flytende, langsomt synkende og hurtig synkende ekstruderte dietter. Ekstrudering reduserte effektiv nedbrytbarhet av

tørrstoff (EDMD) og stivelse (ESD) *in sacco* sammenlignet med ekspandering. Reduksjonen i ESD var størst for dietten med høyest WSI. Effektiv nedbrytning av protein (EPD) var signifikant forskjellig for alle diettene ($P<0.05$) og lavest ved ekstrudering med dampinjeksjon. I overensstemmelse med ESD målt *in sacco*, tenderte fordøyeligheten av stivelsemålt *in vivo* ($P=0.08$) til å være laver for dietten ekstrudert med vannkjøling, som også hadde høyest WSI, sammenlignet med den ekspanderte dietten. Dietten ekstrudert med dampinjeksjon viste nominelt laveste EPD sammenlignet med de andre diettene, i samsvar med EPD målt *in sacco*. Det var ingen effekt av diett på totalfordøyelighet av tørrstoff, stivelse eller protein.

Ekstrudering kan brukes til å endre nedbrytningshastigheten av næringsstoffer i kraftfôr til melkekyr. Det er mulig å påvirke fordøyeligheten i vom uten å forandre totalfordøyeligheten, noe som indikerer muligheter for økt fordøyelse av næringsstoff i tynntarmen.

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List of Abbreviations

g	**gram**
kg	**kilogram**
^{o}C	**degree Celsius**
mm	**millimeter**
cm	**Centimeter**
m	**meter**
d	**day**
µm	**micrometer**
min	**minute**
ml	**milliliter**
L	**liter**
sec	**second**
mmol	**millimole**
N	**Nitrogen**

1. Introduction

Barley is a commonly used grain in the diets for ruminants as an energy source due to its high starch content. Soybean meal (SBM) is commonly used as a protein supplement in beef and dairy cows owing to its good amino acid balance and high availability of protein (Lin and Kung, 1999). Microorganisms in the rumen are crucial to feed digestion where considerable amounts of starch and protein are degraded (Plaizier et al., 2008), whereas feed that escapes ruminal degradation (rumen undegraded feed) is digested and absorbed in the small intestine together with microbial protein and other nutrients from the feed.

Fermentation of nutrients in the rumen and transformation of dietary protein to microbial protein reduces energy utilization (Chalupa, 1975). Hence, the nutrient supply is often not sufficient for dairy cows to have high peak milk production (McCarthy et al., 1989). In addition, rapid fermentation of starch in the rumen is related to reduced efficiency in microbial protein synthesis and increases the risk of subacute ruminal acidosis (SARA) (Hackmann and Firkins, 2015). Therefore, feed utilization must be enhanced in order to achieve maximum nutrient supply for high yielding dairy cows.

Shifting the site of digestion of protein and starch from the rumen to the small intestine has proven to be effective in raising nutrient supply (Larsen et al., 2009) and in alleviating rumen acidosis (Beauchemin et al., 2003). The energetic efficiency of starch digested small intestinally is higher as compared to ruminal and hind gut fermentation (Harmon and McLeod, 2001). Moreover, efficiency of nitrogen utilization is improved when the supply of nitrogen fractions passing to the small intestine is increased (Ipharraguerre and Clark, 2005). Factors that contribute to shifting ruminal degradation of nutrients to the small intestine, are low rate of degradation in the rumen (Doiron et al., 2009) and high passage rate of nutrients from the rumen (Ørskov and McDonald, 1979). Studies with respect to decreasing ruminal starch and/or protein degradation and thus increasing rumen escape are widely reported, using heat processing, i.e., toasting, steam flaking (Offner et al., 2003) and expanding (Prestløkken, 1999). However, the report with respect to altering the rate of rumen outflow (passage) of feed pellets is scarce.

Offer and Dixon (2000) claimed that functional specific density has effects on rumen outflow rate of pellets. Extruder processing can be used to produce feed pellets with specific functional properties, such as high water stability and sinking velocities (Sørensen, 2012). Compared with

extruded pellets, conventional feed pellets for cows have low water stability and high bulk density, indicating fast disintegration and rapidly sinking in the rumen (Larsen et al., 2009).

Thus, extruder processing was employed to produce concentrates with high water stability and sinking characteristics ranging from floating, slow sinking to fast sinking, owing to different bulk densities. The hypothesis was that the extruded concentrates would be degraded to a lesser extent because of high water stability as compared to conventional concentrates with low water stability, and that the slow sinking extruded concentrate would be flushed out of rumen at a higher rate than the floating or rapidly sinking feed. By doing so, the rate of degradation and the rate of passage would be manipulated, thereby shifting site of digestion of nutrients from the rumen to the small intestine.

The objectives of the study were to investigate the effects of extruder processing on (1) functional physical properties of the diet defined as, bulk density, hardness, sinking velocity and water stability index, (2) ruminal degradation characteristics of DM, starch and protein evaluated *in sacco* and (3) rumen fermentation patterns, rumen and total tract digestibility of starch and protein evaluated *in vivo*.

2. Literature review

2.1 The raw ingredients

2.1.1 Barley

Barley (*Hordeum Spp*.) is a cereal derived from annual grass Hordeum Vulgare, characterized by a thick fibrous coat, a high level of β-glucans and simply-arranged starch granules (Nikkhah, 2012). In terms of quantity produced, barley is ranked fourth in the world after wheat, rice, and corn (Jadhav et al., 1998). In Europe barley is the most commonly cultivated cereal grain and is widely used in animal feed. Owing to the local growing conditions, barley has been the main concentrate in the diets of ruminants in Norway, primarily used as an energy source due to the large proportion of starch it contains.

Structure of barley grain

The anatomical structure of a barley kernel is illustrated in Figure 1. The major parts of barley grain include covering layers, the endosperm and the embryo. The hull, lying on top of the pericarp, is the outermost layer of the kernel, which is resistant to microbial utilization of starch in the rumen (Dehghan-Banadaky et al., 2007). The pericarp is developed from ovary walls and acts as a protective tissue over the whole kernel. The endosperm consists of the starchy fraction, accounting for 75% of the total kernel weight, and the aleurone layer which is comprised of protein, lipids, vitamins and minerals (Evers and Millar, 2002). During germination, the starchy endosperm is served as a nutrient source for the growing embryo. Starch granules are embedded in a matrix of storage proteins. The surrounding cell walls of the starch granule contain mixed-linkage (1-3, 1-4)-β-$_D$-glucans (β-glucans) and arabinoxylan at a ratio of 75:25 (Woodward et al., 1988).

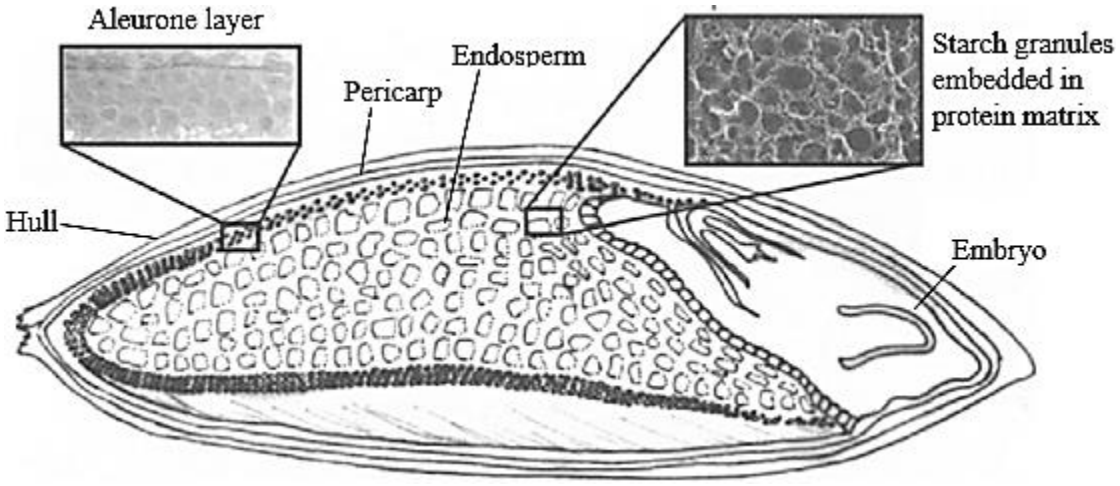

Figure 1. Schematic longitudinal section of a barley grain. Adapted from Fox (2009).

Chemical composition and nutritional value of barley

Many types of barley exist due to variable growing regions and genotype modifications. It is important to understand the type of barley for its use in animal diets and the potential consequences it might have. In addition, the considerable dissimilarities of chemical composition has been observed among different cereal grains, especially the content of starch and thus rumen fermentation patterns (Silveira et al., 2007). The chemical composition of barley compared with other commonly used cereals is shown in Table 1.

Table 1. Chemical composition of barley compared with other cereals.

Nutrient (g/kg as-is basis)	Barley	Hull-less barley	Corn	Wheat	Sorghum	Rye
Dry matter (DM)	904	864	874	890	890	890
Starch	570	650	720	770	720	620
Crude protein (CP)	115	132	88	135	110	121
UCP[1], g/kg CP	280	350	500	250	550	200
NDF[2]	181	120	108	118	161	180
ADF[3]	60	20	30	40	90	100
Fat	19	20	38	22	29	15
Ash	23	19	14	17	18	19
Lysine	4.3	5.0	2.1	3.5	2.7	4.0
Methionine + Cystine	4.2	5.6	3.0	5.1	3.0	3.6
Tryptophan	1.8	1.5	0.9	1.5	0.9	1.4
NE_L[4], MJ/kg	7.2	7.3	7.4	7.6	6.8	7.2

[1] Undegradable CP.

[2] Neutral detergent fiber.

[3] Acid detergent fiber.

[4] Net energy for lactation (NE_L) of barley varies (e.g., 6.3-7.9 MJ/kg) depending on dietary inclusion rate and processing method. Adapted from Nikkhah (2012). Data from Huntington (1997).

Starch is the most abundant component of barley grain, constituting 50-60% of DM (Nocek and Tamminga, 1991). Although starch content in barley is slightly inferior when compared to that in other cereals, the effective ruminal degradability of starch is quite high (80.7-84.6%), being somewhat lower than that in wheat (88.1-88.3%) and oats (92.7-94.0%) (Herrera-Saldana et al., 1990, Huntington, 1997).

Barley starch involves a mixture of large, lenticular granules (10 to 25 µm in diameter) and small, irregular-shaped granule (<10 µm) (Jadhav et al., 1998). The starch granule is composed of two types of molecules: amylose and amylopectin (Santana and Meireles, 2014). Amylose contains about 99% of α-1,4-D-glucose polymers arranged linearly (Parker and Ring, 2001). Amylopectin, considerably more abundant (700-800 g/kg) in starch granules, consists of 95% α-1,4 links and 5% α-1,6 links located at the branching point of molecules (Stevnebø et al., 2006). Figure 2 shows the

structure of a starch granule formed by two types of alternating growth rings, referred to as amorphous lamella and crystalline region, respectively. Amylopectin molecules form double-helices, which crystalize and contribute to crystalline nature of the starch granule, whereas the amorphous lamella is incompact and is composed mainly of branching points of amylopectin (Buléon et al., 1998). Amylose molecules are randomly interspersed among amylopectin. Crystalline parts are more resistant to acid hydrolysis and enzymatic attacks compared to amorphous areas which are considered to be more susceptible (Buléon et al., 1998).

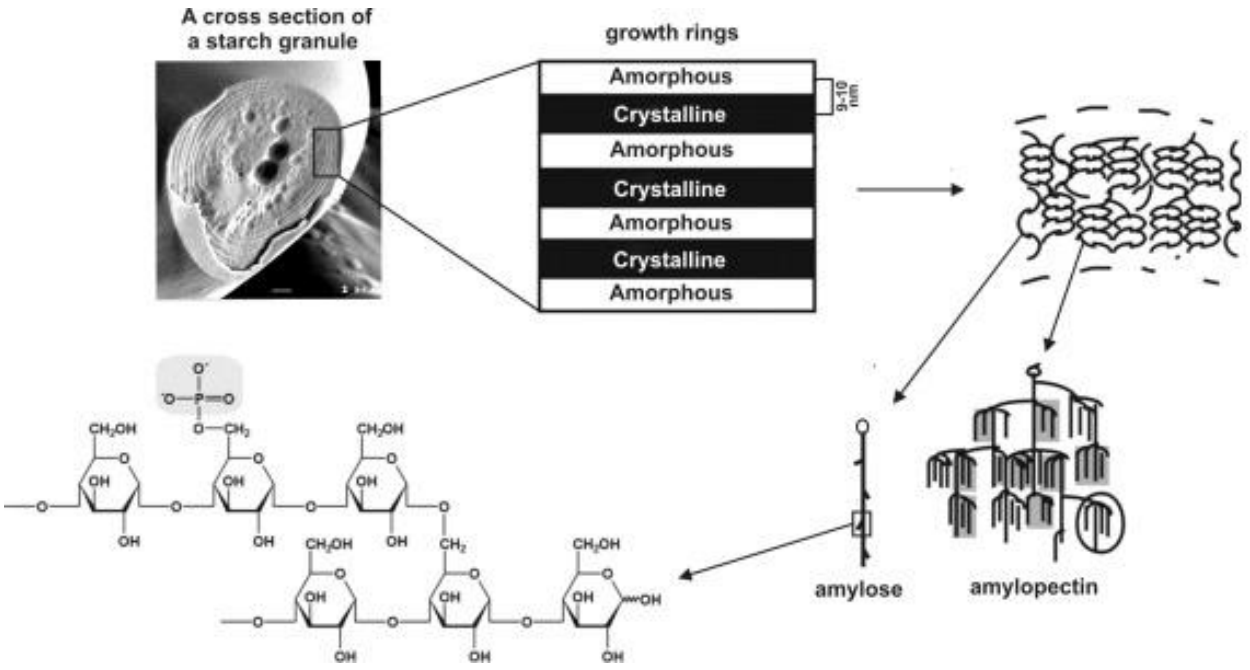

Figure 2. Schematic representation of the structure of a starch granule (Nazarian-Firouzabadi and Visser, 2017).

Barley cultivars with various amylose content and different starch granule size affect starch degradation. Varieties such as waxy barley starches have very little amount of or no amylose, while high-amylose varieties contain >700g amylose/kg. Gómez et al. (2016) and Stevnebø et al. (2006) concluded that barley cultivars containing low amount of amylose had a higher degree of starch hydrolysis in vitro than those with normal or high amylose level. They also claimed that small starch granules were degraded at a higher rate than large granules due to smaller granules possessing larger surface area.

The amount of CP in barley is prominent, especially the hull-less barley containing 132 g/kg crude protein, which is a bit less than the amount contained in wheat (135 g/kg). Moreover, barley is rich in lysine, methionine and cysteine, and tryptophan in comparison with other cereals. These information imply that barley has potential to meet protein requirements of high-producing ruminants (Nikkhah, 2012).

Barley contains large amounts of non-starch polysaccharides (NSP) as compared to other cereals. The NSP exhibit viscous property in the digestive tract. The high gut viscosity hinders interaction between digestive enzymes and nutrients and thus reduces digestion and absorption of nutrients. Thereby, NSP are generally considered as anti-nutritive factors for monogastric animals (Choct, 1997). NSPase enzymes, such as β-glucanase and β-xylanase, are supplemented in feed to degrade NSP into smaller fragments, down-regulate gut viscosity and improve digestibility (Choct and Annison, 1992, Coppedge et al., 2011). Contrary to monogastric animals, ruminants are not affected by NSP because microbes in rumen can degrade and utilize them as energy sources.

Fat content in barley is relatively low when compared to that in other cereals. Normally barley has fat in the range of 2% to 3%, with genotypic variations containing up to 7%. Linoleic and palmitic acids are the major fatty acids found in barley. Most of fat in barley is stored in endosperm which results in the formation of lipid-starch complexes that provide dramatic adhesion between molecules (Vasanthan and Bhatty, 1996). The complex decreases starch swelling during processing, in consequence, reducing enzymatic digestion of starch (Crowe et al., 2000). It is shown that the complex is formed between lipids and amylose in the amorphous zone (Morrison et al., 1993).

2.1.2 Soybean

The soybean (*Glycine max*) is one of the most valuable agricultural commodities because of its unique chemical composition (Banaszkiewicz, 2011). Among cereal and other legume species, it has the highest protein content and its amino acid composition is comparable to composition of meat proteins. Beef and dairy cattle require amino acids both from microbial and dietary protein. Soybeans can be formulated into any type of forage-based diet and provide high quality protein and energy (Ishler and Varga, 2000).

Structure of soybean seed

The soybean seed is composed of a seed coat or hull (8%), two large cotyledons (90%) and two minor parts, the germ and hypocotyl-axis (2%) (Bair, 1979). Figure 3 shows cross section of mature soybean hull and part of the cotyledon. From the outermost to the inner layer, the seed coat is comprised of palisade cells, hourglass cells and parenchyma. Aleurone and compressed cells constitute most of the endosperm. Under the endosperm, palisade-like cells characterize the nutritionally important layer, cotyledon, containing protein and oil. Lipid body is found to attach itself onto protein body in the whole soybean (Liu, 2012).

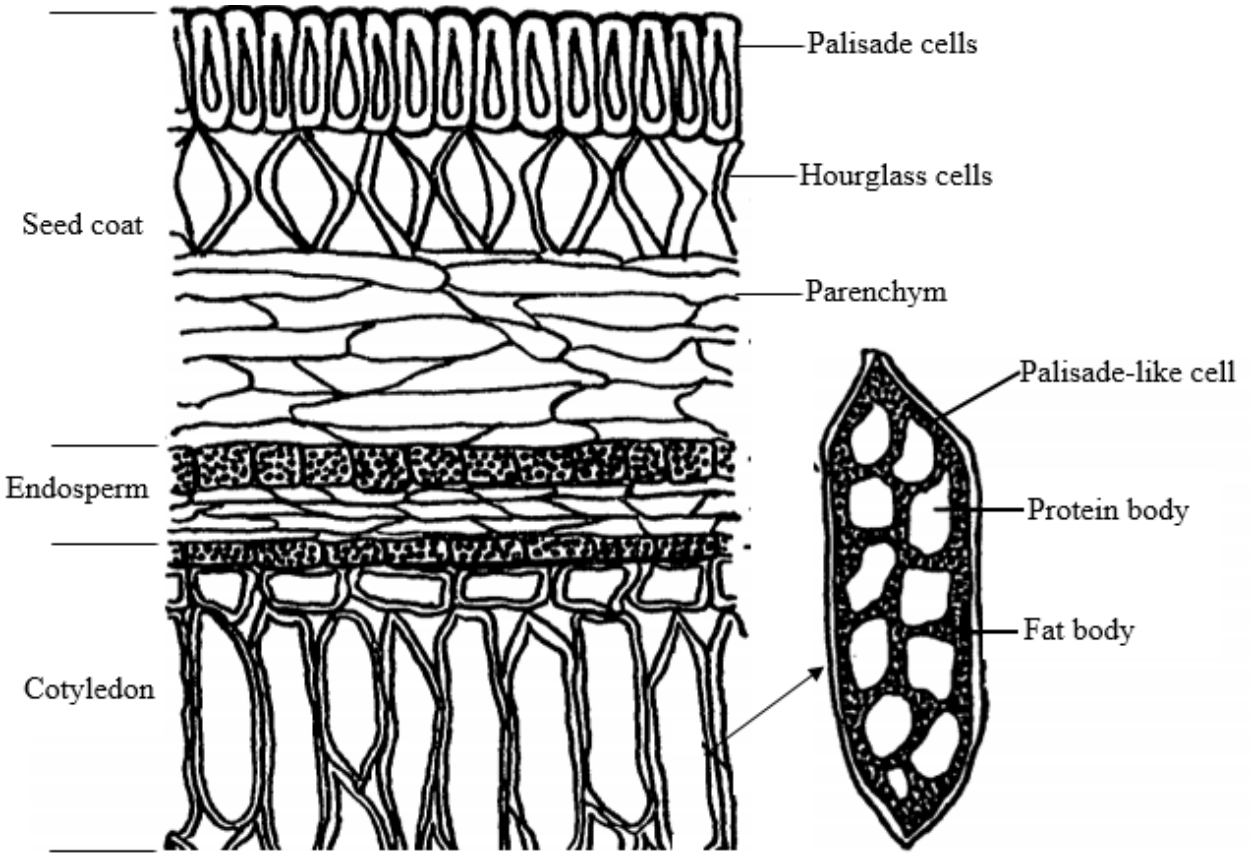

Figure 3. Cross section of mature soybean hull and part of the cotyledon. Adapted from Bair (1979).

Chemical composition and nutritional value of soybean meal

Whole soybeans contain about 20% fat and 36% protein by weight (Hassan, 2013). The whole soybeans are subjected to a series of processing for producing SBM, including cracking, softening, flaking, solvent extraction by hexane and toasting at 105 ^{o}C for 30 min. The end product contains

48% or 45% (with hull included) of protein on dry matter basis. Table 2 shows the variation of chemical composition from full fat soybeans to solvent-extracted SBM.

Table 2. Chemical composition of soybeans and solvent-extracted soybean meal. Adapted from Willis (2003).

Nutrient (g/kg as-is basis)	Soybeans	SBM (solvent-extracted)
Dry matter	908	880
Crude protein	380	480
Crude fat	190	25
Crude fiber	55	42
Starch	< 10	< 10
Neutral detergent fiber (NDF)	226	80
Available phosphorus	2	2
Calcium	2	3

Full fat soybean meal is a favorable source of energy and fatty acids. Of the lipid faction in the soybean, the content of polyunsaturated fatty acids (linoleic and linolenic) and unsaturated (oleic acid) account for a large proportion (Banaszkiewicz, 2011). With fat removal, the protein content of SBM elevates greatly.

SBM is an excellent vegetable protein source considering of its quantity as well quality. Compared with other oilseeds, the content of crude protein is superior (Table 3). The protein of SBM contains substantial quantity of lysine, but the protein value is compromised by methionine and cystine content. In addition, the nutritive value of SBM is constricted by protease inhibitors – kunitz and Bowman-Birk, which impede digestion of nutrients through inhibiting the activity of trypsin and chymotrypsin (Winiarska-Mieczan, 2007). Moreover, lectins are proteins that bind to carbohydrate and thus interfere with digestion. However, adequate heat processing inactive these inhibitors, i.e. toasting of soybeans.

Table 3. Comparison of various oilseeds on dry matter content, crude protein and neutral detergent fiber (g/kg as-is basis). Adapted from (Machmüller et al., 2000, Willis, 2003).

Oilseed meals	Dry matter	Crude protein	NDF
Soybean meal	880	480	80
Canola meal	940	350	260
Cottonseed meal	920	390	300
Peanut meal	900	460	80
Sunflower meal	930	300	450

The carbohydrates in the soybean consists of approximately 10% free sugars (sucrose, raffinose and stachyose) and 20-30% NSP, in which roughly 90% are pectic polysaccharides and the remaining are cellulose (Choct, 1997). The soybean contains very little of or no starch. The neutral detergent fiber (NDF) content in SBM is relatively low, when compared to that in soybeans (Table 2) and to other oilseed meals, such as canola, cottonseed, sunflower meals (Table 3).

2.2 Digestive physiology of ruminants

2.2.1 Rumen physiology

Ruminants have evolved a digestion system which makes them capable of digesting β-linked feedstuffs, such as cellulose. This system employs microbial fermentation to digest nutrients in feedstuffs before they are exposed to the animals' own digestive enzymes. The stomach (Figure. 4) of the ruminant consists of four parts, the rumen, the reticulum, the omasum and the abomasum. The three first are forestomaches with microbial fermentation and the last one (abomasum) is comparable to the stomach of monogastric animals. The two first compartments, often considered as reticulo-rumen, account for 85% of the total capacity of the stomach in the adult ruminant.

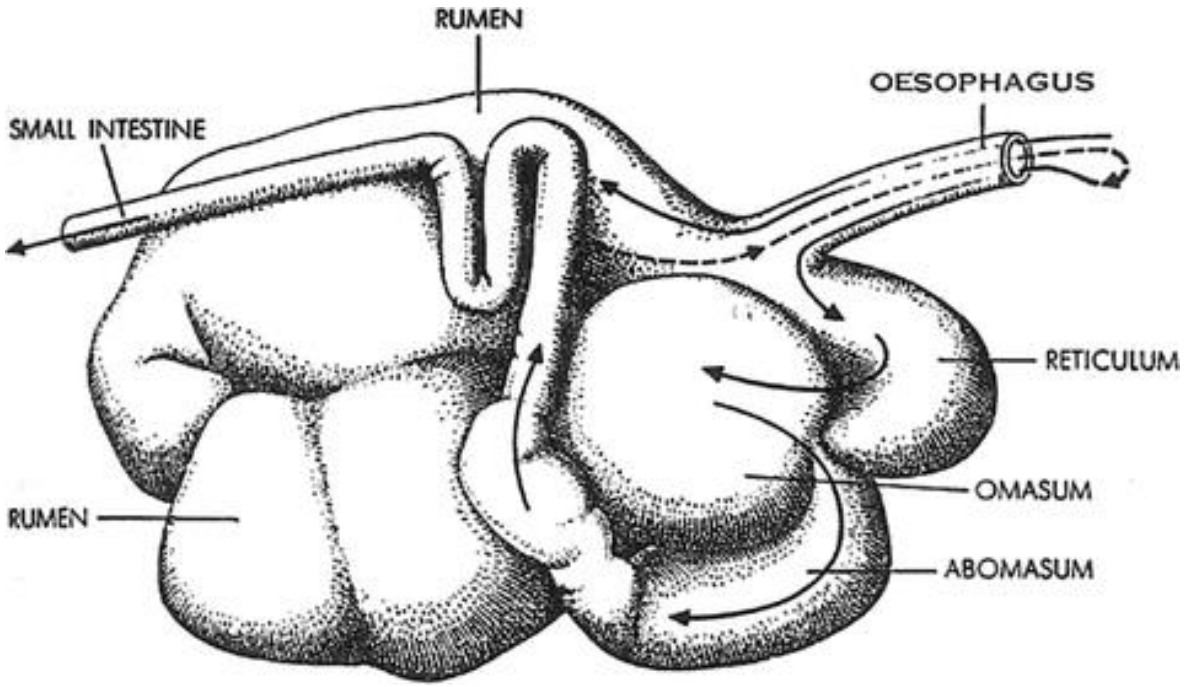

Figure 4. Diagrammatic representation of the stomachs and flow of digesta (McDonald, 2011).

Digesta in rumen exist in two main phases, the upper layer of coarse solid particles and the lower layer of liquid, with water averaging 850-930g/kg of rumen contents. The coarse and large particles are further broken down through rumination. Ruminal digestion conducted by microbes yield principally volatile fatty acids (VFA), microbial cells and gases, which are partitioned to rumen-wall absorption, pathway to abomasum and small intestine together with undegraded feeds, and loss by eructation, respectively. Rumen microorganisms are divided into three species, bacteria, protozoa and fungi. The bacteria are subdivided into amylolytic, cellulolytic, proteolytic and methanogenic types. Based on sites of action, microorganisms either exist in liquid or attach to particles or adhere to the surface of epithelium (McDonald, 2011).

2.2.2 Digestion of starch

The digestion of starch is associated with amylolytic bacteria, fungi and protozoa. Enzymes secreted by bacteria degrade starch into smaller polymers, carried out by α-1,4 and α-1,6 endo- and exo-amylases (Huntington, 1997). Then, maltase and 1, 6-glucosidase perform degradations on the corresponding counterparts to produce glucose or glucose-1-phosphate. After that, the key intermediate, pyruvate, converted from glucose-1-phosphate is transformed, by several routes, into acetate, propionate and butyrate (VFAs) as well as gases (Figure. 5).

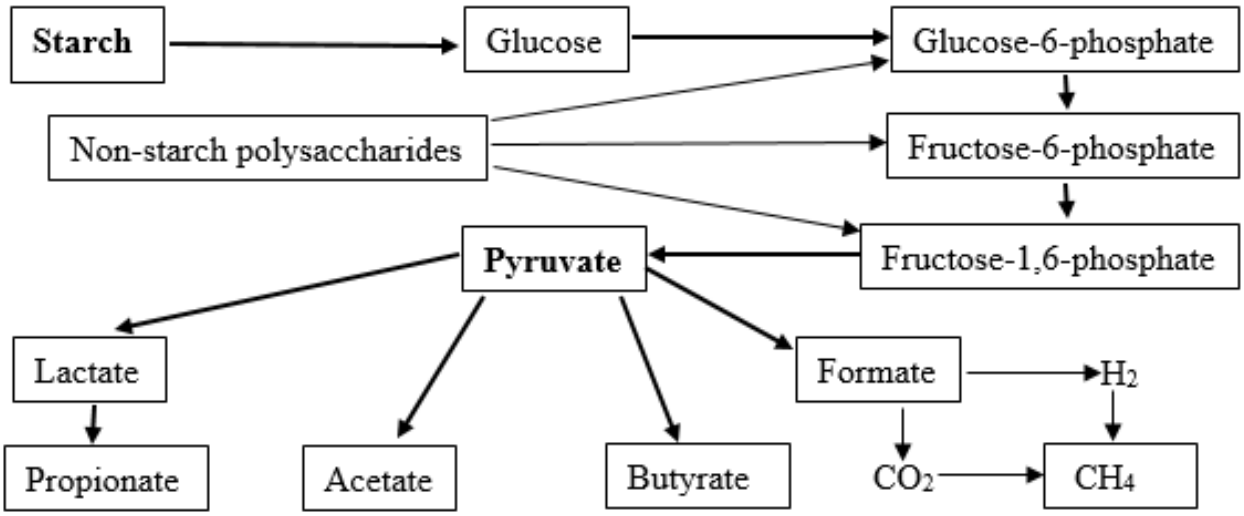

Figure 5. Digestion of carbohydrates in the rumen. Modified from (McDonald, 2011).

As starch is rapidly fermented, the production of lactic and propionic acids causes the acidity to increase and the elevation of acidity suppresses cellulose-digesting bacteria. Large quantities of grain provided in cow's diet often leads to a more acidic environment, termed acidosis. Common forms of acidosis are known as subacute ruminal acidosis or SARA (pH: 5.0-5.5) and acute ruminal acidosis (pH below 5.0) (Krause and Oetzel, 2006). Cows suffering from acidosis are associated with laminitis and other health problems (Plaizier et al., 2008). Protozoa can engulf the starch particles and thus affect its availability to other microbes. This process, combined with the engulfment of lactic acids by protozoa, aids stabilization of rumen pH. The normal rumen pH is maintained at 5.5-6.5, attributed to buffering effect of phosphate and bicarbonate contained in saliva, and most importantly the fact that VFA are absorbed through rumen wall. Except the starch digested in the rumen by microbes, the undigested starch in the feed is removed from rumen through the reticulo-omsal orifice to the abomasum and the small intestine. Starch escaping rumen digestion would be digested by host animals' enzymes and absorbed in the small intestine or reabsorbed as VFA via fermentation in the large intestine. Eventually, starch not digested by the animal would be excreted in feces.

To increase the efficiency of starch and reduce the risk of ruminal acidosis, the increase of the amount of rumen undegraded starch is considered to be an efficient alternative (Zebeli et al., 2010). Huhtanen and Sveinbjörnsson (2006) reviewed that compared to ruminal digestion, starch digestion in the small intestine indicates better energetic efficiency thanks to the reduced heat loss

and methane production in rumen. However, the shifting of starch digestion from the rumen did not imply an increase in the small intestine but led to an elevated fermentation in the hindgut and lower total tract digestibility (Larsen et al., 2009). It might be due to the limited enzymatic digestion for rumen undegraded starch in the small intestine and thus increased fermentation in the hindgut where VFA are absorbed but microbial matter is excreted in the feces. Nevertheless, individual studies are unable to conclude comprehensively. The source of feedstuffs, formulation and feed processing all together influence the outcome of feed utilization.

2.2.3 Digestion of protein

Figure 6 shows the pathways of nitrogenous compounds in rumen. Rumen microorganisms degrade protein into peptides and amino acids. Furthermore, some of the amino acids in rumen undergo deamination to form ammonia, carbon dioxide and organic acids, such as isobutyric and isovaleric acids. The ammonia plays a key role in ruminal protein digestion. When the amount of ammonia is low because of the deficient protein supply, the breakdown of carbohydrates will be depressed. Conversely, if ammonia quantity is too high to exceed the optimum concentration, it will be absorbed through rumen wall to blood and conveyed to liver where it is converted to urea. Some of urea return to rumen through saliva or rumen wall, but most of it are wasted in urine. Rumen proteolytic bacteria and protozoa utilize ammonia together with the peptides and amino acids to synthesize microbial proteins. A fraction of microbial proteins is broken down to produce nitrogen (N) (recycled in the rumen). Another fraction along with dietary undegradable protein is digested and absorbed in the abomasum and the small intestine.

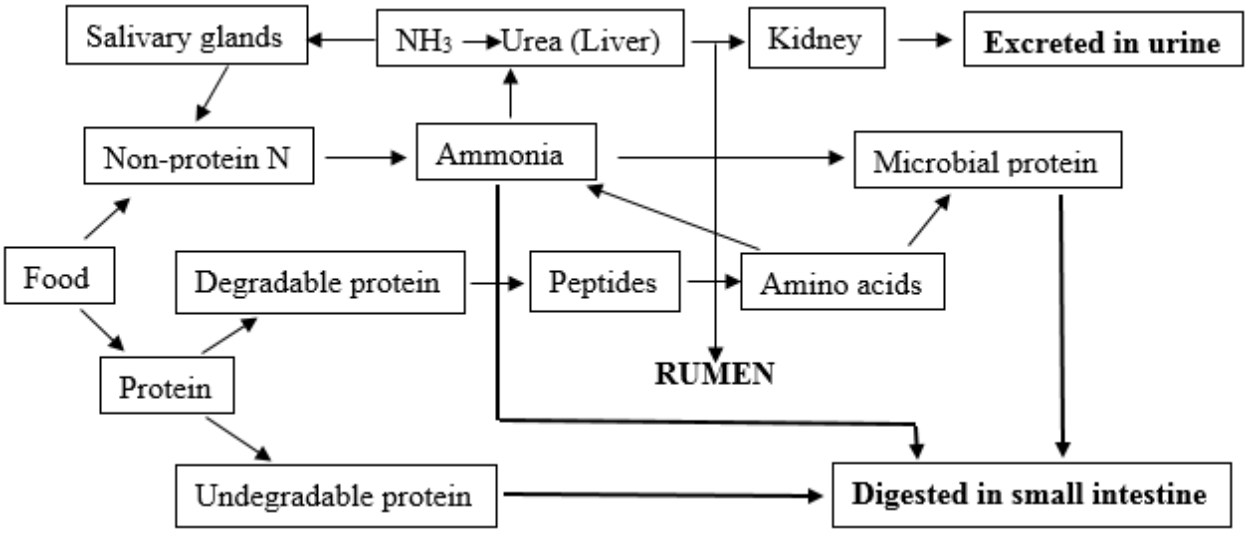

Figure 6. Digestion of nitrogenous compounds in the rumen. Modified from (McDonald, 2011).

A great proportion of protein reaching the small intestine will be microbial protein synthesized from rumen degraded protein and the rest contains mainly rumen undegradable protein from the feed (McDonald, 2011).

For ruminants, the amount of amino acids from microbial protein and dietary rumen undegradable protein absorbed in the small intestine determine the protein value of a feed stuff (Prestløkken, 1999). In addition, reabsorbed endogenous amino acids contribute a minor fraction of the protein value. To improve the protein value of feedstuffs for high-producing dairy cows, attempts have been made to protect proteins from rumen fermentation so as to improve the small intestinal digestion. For example, the xylose-treated SBM (SoyPass), manufactured by adding xylose under heat and elevated moisture, reduced effective rumen degradability of total amino acids to 29% compared to 53% for SBM, despite the decrease of intestinal digestibility for some acids (Harstad and Prestløkken, 2000). This is consistent with Nobar et al. (2009) who reported that xylose-treated SBM (SoyPass) significantly decreased rumen degradability of CP for SBM.

2.3 Feed processing

Feed processing is related to any procedure undertaken to change the physical and/or chemical characteristics of an ingredient to increase its nutrient availability, or to improve its functional quality. The techniques applied in feed production include grinding, weighing, mixing,

conditioning, pelleting, expansion, extrusion and drying or cooling. In this chapter, the grinding, conditioning, pelleting, expansion and extrusion will be described.

2.3.1 Feed processing techniques

Grinding

Grinding accomplishes particle size reduction of feed materials so that it exposes greater surface area for digestion, and improves ease of the subsequent treatments, such as mixing and pelleting (McEllhiney, 1994). The typical grinders are the hammer mill (Figure 7a.) and the roller mill (Figure 7b.). The working principle of the hammer mill is to impact the materials by the hammers. As the materials fall into the grinding chamber by gravity, the ganged hammers mounted on the bolt axis driven by a rotating shaft strike the materials. This aggravates collisions of materials with hammers, the chamber and the particles, whereby the materials are shattered to a certain size to pass through a given sieve opening. An air assist system, as an integral part of most hammer mills, facilitates the grinding efficiency by a fan that sucks through the hammer mill the same direction as the feed flows. The roller mill is characterized by two pairs of counter-rotating rolls which crush the feed as it passes between. Each pair of rollers is fitted such that one is placed fixed and the other one is adjustable to determine the extent of grinding. It is of importance that the material flow is even and passes through the whole width of the roller.

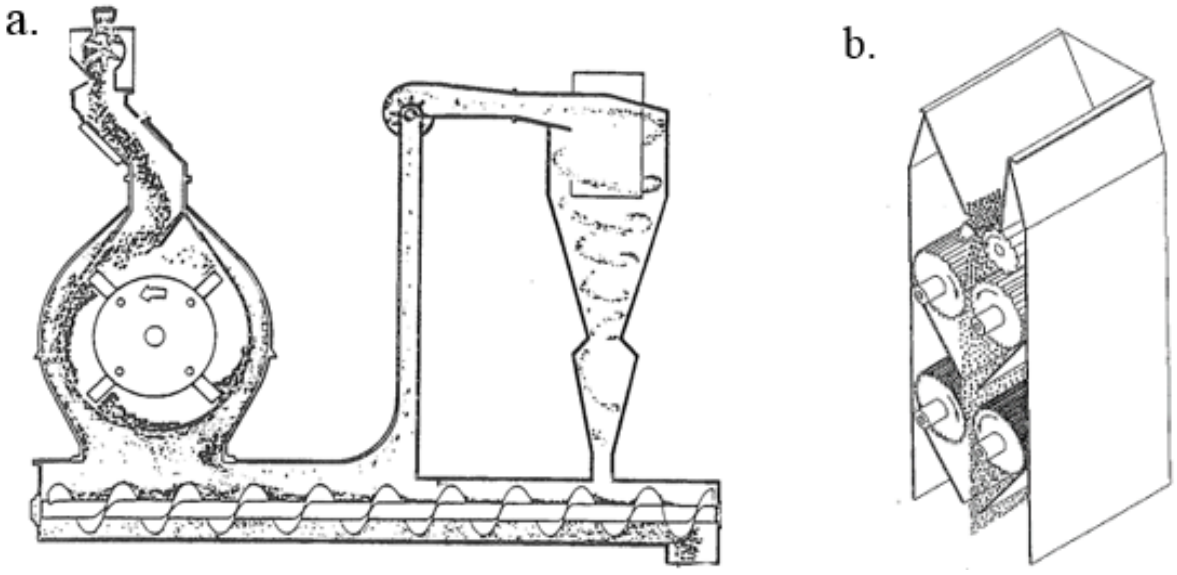

Figure 7. Schematic figure of a hammer mill and a roller mill Svihus (2014).

Conditioning

Conditioning is the process where steam is applied during the contant agitation of feed mash in a cylinder. The conditioner is used before the feed mash to undergo pelleting, expanding and extruding and thus the purpose of conditioning is to preheat and moisten the materials such that the following process becomes more lenient. The long-term conditioner (Figure. 8) consists of two cylinders, of which the upper one allows steam addition and agitates the feed by paddles fixed on a rotating shaft, of which the lower one with a conveying screw and a heated wall icreases retention time and thus ensures the feed at desired temperature. When the temperature is above 81 ^{o}C and lasts for more than 30 sec, salmonella would be killed.

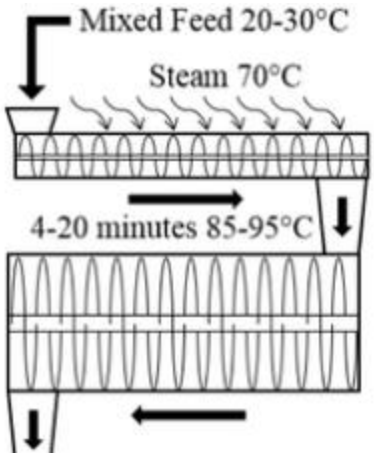

Figure 8. Schematic figure of a long-term conditioner (Sibanda and Ruhnke, 2017).

Care should be taken that the steam added does not contain condensed water in order to assure a maximum heat transfer. As a rule of thumb, 10 ^{o}C increase in mash temperature needs 0.7% of water in the form of steam.

Pelleting

Pelleting provides a means of molding feed mash into larger particles and is accomplished through a mechanical process combined with heat, moisture and pressure (McEllhiney, 1994). The pellet press is placed right posterior to the conditioner so that the temperature loss is minimized when the conditioned materials enter the pellet press. The temperature is normally maintained at above 80 ^{o}C. Feed mash falling into the pellet press will be forced through a thick metal plate having cylindrical holes, a so-called die (Figure. 9a & b). As the mash falls between the roller rotating along the inner surface of the die, the pressure caused by the compression between the roller and

the die squeezes it into the holes in the die. The resulting pressure combined with the elevated temperature from frictions causes the particles to be glued together. While the pellet appears out of the die, in case the pellet is too long, a knife is employed to cut it off. To avoid the blocking of the hole due to wear, the opening of the hole is funnel-shaped to ease flow.

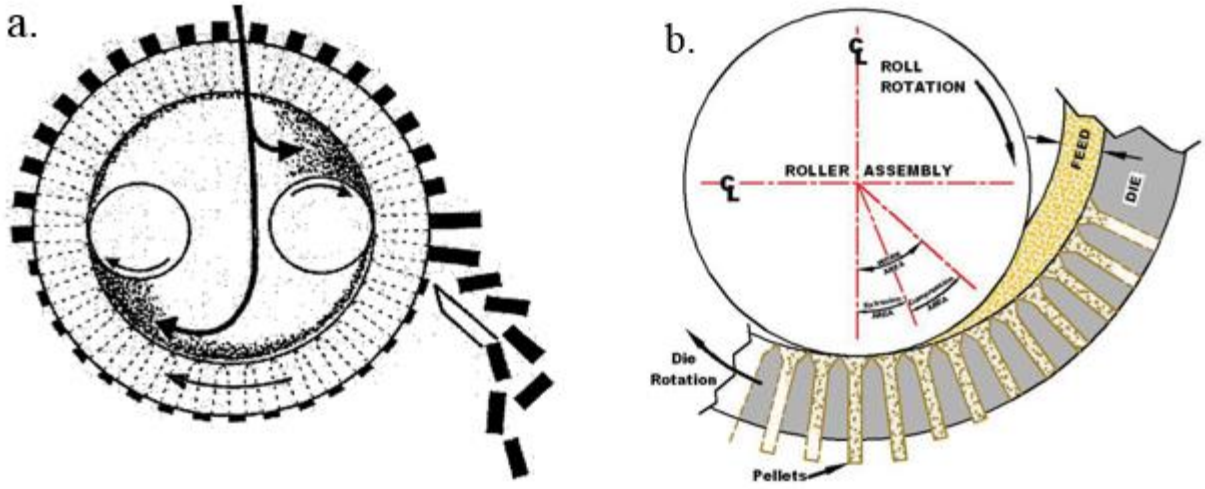

Figure 9. Designs of a ring-shaped die (a.) of a pellet press and the working principles (b.) (Svihus, 2014).

Benefits associated with pelleting include hygienic conditions of the feed, improved flowability, and elimination of segregation problems (Huang et al., 2015).

Expanding

Expanding is a method that the feed material is subjected to high tempeature up to 130 ^{o}C for a short time (Prestløkken, 2013). One of the most common types of the expander is the annular gap expander (Figure. 10).The feed materials enter the cylinder through an inlet gate, whereafter a screw rotating at a high rate conveys them towards a resister in the outlet gate of the expander, during which steam is added (Prestløkken, 2013). Since the speed is quite high, the sudden stop by the cone-shaped resister causes the build-up of pressure and heat in feed materials. Once the materials come out of the outlet, the immediate pressure drop and water evaporation result in the expansion of the feed pellet prior to experiencing pelleting. After pelleting, the materials are conveyed to a cooler for cooling. As an advantage, the expander permits to process relatively large quantity of feed at a low cost (Prestløkken, 2013).

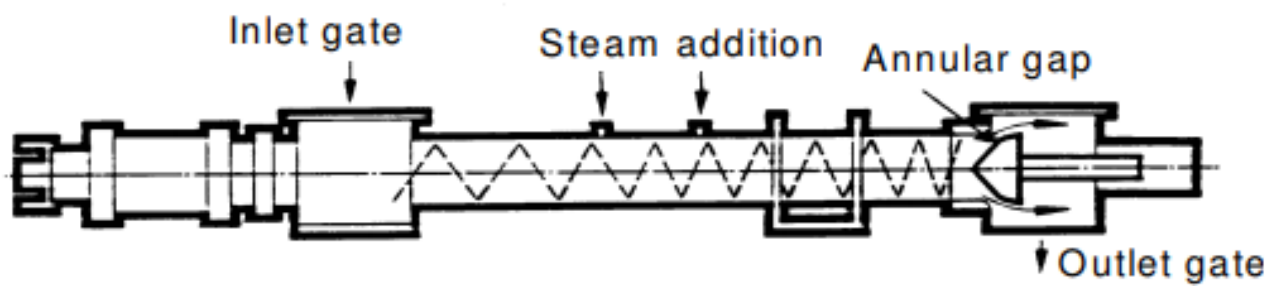

Figure 10. Schematic figure of an annular gap expander (Prestløkken, 2013).

Extrusion

Extrusion cooking involves high pressure, heat, moisture and shear forces, which causes clear changes in physiochemical and functional properties of the feed (Sarawong et al., 2014). The twin-screw extruder (Figure. 11) consists of two screws wrapped around closely by a mental wall, the nozzles on the extruder wall for steam and water addition as well as a head, called die, modelling the extrudate. In similarity with expander, the feed pellet is expanded due to pressure drop and is cut off by a knife. The extruder screw is comprised of a variety of screw elements, possessing different functions, such as conceying (1,2), kneading (3) and cooking (4,5). Different from expander processing, extrusion cooking allows more water addition and heat transfer to modify the feed structure. Morken et al. (2012) showed that extruded feeds for fish improved the availability and utilization of nutrients and stimulated feed intake due to a favourable feed structure. Extrusion cooking has crucial effects on physical quality of the feed with regard to hardness, durability, sinking velocity and water stability (Sørensen, 2012). The physical quality differs with diet formulation (Kraugerud and Svihus, 2011), extruder configuration (Sørensen et al., 2010) and processing parameters (Sørensen et al., 2011).

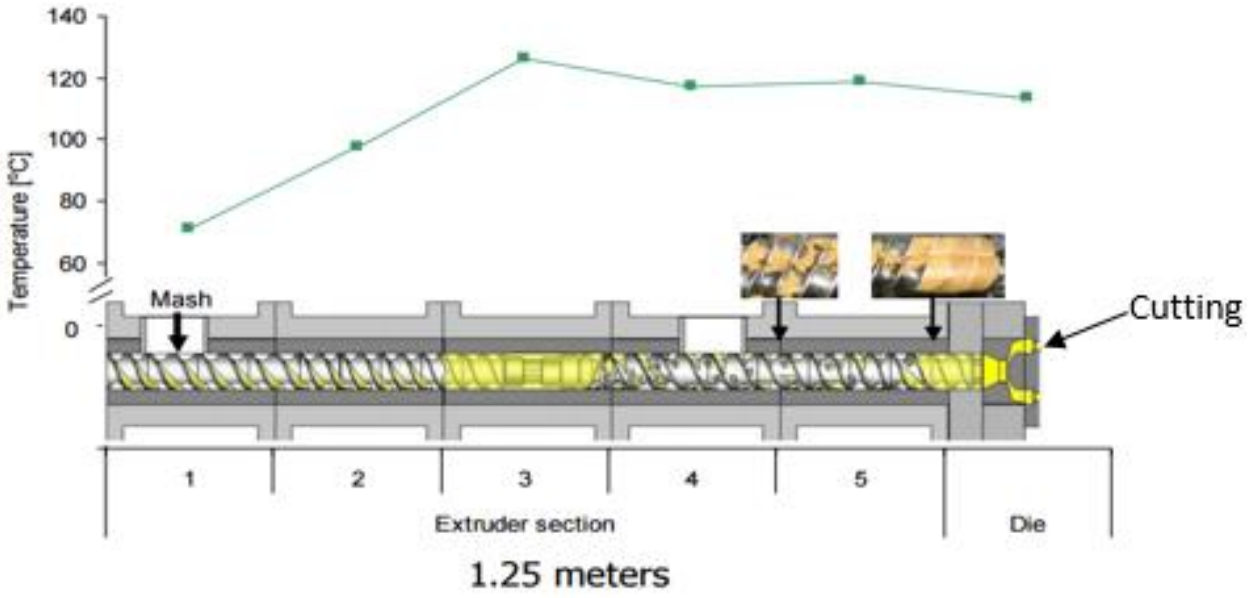

Figure 11. Schematic figure of a twin-screw extruder (Svihus, 2014).

2.3.2 Effects of heat treatment on structural alterations of starch and protein

Heat is applied in conditioning, pelleting, expanding and extrusion cooking processes. Gelatinization of starch occurs upon heating to above 80 ^{o}C in excess water (Svihus et al., 2005). During heating, as the semi-crystalline region is more resistant to water because of the condensed arrangement of molecules, water absorption first occurs in the amorphous region, causing the swelling phenomenon. Swelling increases stress at the interface between amorphous and semi-crystalline space. At a certain point, the stress is big enough to disrupt the hydrogen bonds between amylopectin in crystalline region. As a result, the molecules break down and swell due to water penetration, indicating gelatinization onsets. The extent of gelatinization differs among different heat treatments. Conditioning and pelleting geletinize the material between 1% to 19%, expanding geletinizes the material more than that saying >20% due to the higher moisture content and heat input, while extrusion cooking could geletinize the material up to 100% owing to the excess of water and the substantial heat transfer (Svihus et al., 2005). Gelatinization causes the amylose molecules to leach into the surrounding water and thus increases material viscosity owing to the solubilized amylose (Hermansson et al., 1995). Such change may contribute to physical quality of the feed through increased binding between particles. In addition, starch becomes more exposed to enzymatic digestion through gelatinization, which is considered beneficial for non-ruminants but is likely to increase the possibility of rumen acidosis due to the rapid degradation of starch.

Retrogradation of starch is defined as the reversible return of a dispersed and solubilized state to a crystalline and insoluble form in an amorphous matrix. Biliaderis (2009) reported that amylose is the main component to facilitate retrogradation. Amylopectin retrogradation is usually negligible because it arises very slowly and uses weeks of storage to proceed (Lii et al., 2004). Retrograded starch is reported to depress digestion through resistance to enzymatic attacks (Eerlingen et al., 1994).

Protein is heat sensitive. When heat is applied, the kinetic energy causes the molecules to vibrate so violently and rapidly that protein loses the quaternary, tertiary and secondary structure because of the disruption of hydrogen bonds. This process is known as denaturation of protein. The denatured protein exposes its hydrophobic bonds which reduces its solubility. Furthermore, severe heat could result in the aggregation of protein molecules, possibly due to the sulfuric cross linkages formed during heating. Expander and extruder processing could induce browning reactions between reducing sugars and amino groups, recognized as Maillard products. The rate and extent of Maillard reactions are dependent on temperature, pH and moisture content of materials (Woodroofe and Cockbill, 2001).

2.3.3 Effects of the processing on nutritional value of the feedstuff

Grinding causes size reduction of the feed. The grinding level has a great influence on the digestibility of nutrients. According to Michalet-Doreau and Cerneau (1991), ruminal nitrogen degradability decreased for barley and soybean when the grinding screen elevated from 0.8 to 6.0 mm. However, Froidmont et al. (2008) claimed that the small (0.5 mm) or the large (6.0mm) screen size led to higher ruminal nitrogen degradation, and that particle size between 2.0-4.4 mm was suggested to decrease the nitrogen degradation in rumen, even though the ingredient they investigated was lupin seed. Therefore, the grinding level needs to be tested during the experiment.

Pelleting may increase gelatinization of starch and denaturation of protein during processing, which results in increased microbial attacks in the rumen and hence the elevated degradability. This claim could be justified by the results from Goelema et al. (1999), who testified that pelleting generally increased total protein digestibility of peas, lupins and faba beans in the rumen as well as intestinal digestibility of rumen undegraded protein and starch. Moreover, pelleting significantly increased ruminal starch digestibilty of these beans. However, in the experiment of Ljokjel et al. (2003), pelleting did not increase rumen degradation of starch in barley, under conditions with

temperature around 80 ^{o}C and low moisture content. The reason for this might be differences in degree of gelatinization of starch, or differences in how processing affected particle size.

Expander processing adds slightly more water and creates fairly more heat to feed materials compared with pelleting. A few studies have shown that expander treatment increased *in vivo* rumen degradation of starch in grains like barley and oats (Harstad et al., 1996, Prestløkken and Harstad, 2001, Tothi et al., 2003). Contrary, expander treatment significantly reduced rumen degradation of starch in barley (Offner et al., 2003), whereas Ljokjel et al. (2003) found reduced rumen degradation of starch in oats but not in barley. With reference to rumen degradation of protein, Prestløkken (1999) found that expander treatment considerably decreased effective protein degradability in barley, oats, SBM, a cereal mixture and to some entent in a protein mixture. In line with that finding, Ljokjel et al. (2003) revealed that expander treatment efficiently protected protein from rumen degradation in barley and SBM. However, expander treatment had effects neither on total ruminal digestibility of protein nor starch, as demonstrated by Goelema et al. (1999). Hence, these conflicting results indicate that the effects of expander treatment on rumen degradation of starch and protein are affected by many factors, e.g., the type of material processed, heat and water added in expander and the type of animal to be fed (Prestløkken, 2013).

The effects of extrusion cooking on rumen degradation of protein and starch are contradicting. Offner et al. (2003) reviewed that extrusion, as compared to pelleting, increased rumen degradation of starch in maize and sorghum. This is reasonable because the relatively higher moisture content and heat transfer give rise to a higher extent of starch gelatinization during extrusion. Moreover, rumen degradation of starch of sources with high fraction of starch resistant against microbial degradation is generally increased (Svihus et al., 2005). Even so, some positive effects have been showen for ruminants. Walhain et al. (1992) found that extrusion markedly reduced ruminal degradation of protein in pea *in sacco*, whereas effective DM degradability was not affected. Griffiths (2004) showed that extrusion significantly lowered effective degradability of DM fraction of SBM. When the extruder was used to produce pellets with low density the extrudate possessed the lowest effective protein degradability as compared to both the feed pelletized and meal (Razzaghi et al., 2016). This is probably due to the favorable binding property between particles at low density.

2.4 Construction of hypotheses

In terms of energetic efficiency and protein value to the dairy cows, the shifting of starch and protein degradation from the rumen to the small intestine has proven to be advantagous over ruminal fermentation (Mills et al., 1999, Harmon, 2009, Hungate, 2013). This shifting could be accompolished by changing the rate of feed digestion in the rumen (Goelema et al., 1998).

The rate of digestion is affected by the pellet structure. The structural modificaton of feed achieved by heat treatment, such as extruding, slowed down the rate of corn-based starch fermentation and reduced ruminal ammonia N without compromising the intestinal digestibility (Shabi et al., 1999). Likewise, Doiron et al. (2009) showed that heating changed the protein structure of α-helix to β-sheet ratio such that rumen-degradable protein decreased and the potential protein supply to dairy cows increased.

Besides this, the rate of passage for particles affects the digeston of the feed (Hansson, 2006). Pellets with different sizes and shapes are first floated in the liquid phase of rumen after ingestion. Cows fed a diet high in grain concentrates have a very small 'floating mat' as all feed pellets sink to bottom of the rumen immediately. Ørskov and McDonald (1979) showed that the higher the outflow rate, the less nutrients are degraded in the rumen. Rumen undegraded protein would be increased when the passage rates of feedstuffs out of the rumen increased (Seo et al., 2006).

Hence, the rumen undegradable fractions of starch and protein could be improved by manipulating the rate of ruminal digestion and the passage of feed out of rumen.

Pellets with high water stability possess enhanced physical integrity with minimum disintegration and nutrient leaching while in water (Ighwela et al., 2014). Extrusion as a continuous cooking system enables raw materials to undergo physiochemical transformations (Ding et al., 2005). It is widely used to produce fish feed with high water stability and/or varying sinking characteristics (Kannadhason et al., 2009). In the present experiment, four concentrates will be used in the animal experiment, three extruded and one expanded concentrate (control). The extrusion cooking was employed to produce the experimental concentrates with high water stability. Meanwhile, steam injection or water cooling conditions were applied during extrusion cooking to make feeds with a spectrum of bulk densities, which resulted in pellets having diverse sinking characteristics, such as floating, slow sinking and fast sinking (Sørensen, 2012).

Therefore, the hypotheses of this experiment were:

1) As compared to the expander treated concentrate, the extruder treated concentrates have higher WSI and thus will be degraded in rumen at a lower rate.

2) The extruded concentrate, which is slow sinking in the rumen combined with high WSI, will pass out of rumen at a higher rate as compared to the floating and fast sinking concentrates.

3. Materials and methods

3.1 Processing of the concentrates

Feed processing was carried out at Fôrtek (Center for Feed Technology) owned by the Norwegian University of Life Science (NMBU). The processing was done in two steps. In the first step, a total of nine extruded concentrates were processed in a pilot study. All concentrates were then subjected to physical property tests in terms of bulk density, hardness, sinking characteristics and water stability index (WSI). Three of the nine extruded concentrates with desired physical properties were selected and processed in the second step to be used in an animal experiment. For the animal experiment, a control concentrate was also produced using expander processing. Thus, concentrates for the animal experiment consisted of three extruded concentrates and one expanded concentrate as control.

Pilot production of concentrates

Each of the nine extruded concentrates was a blend of barley and solvent-extracted soybean meal (SBM) at a ratio of 65:35. All ingredients were ground by a hammer mill (E-22115 TF, Muench-Wuppertal, Germany) to pass either 2 or 4 mm screen size. Barley and SBM mash were mixed by a twin shaft paddle mixer (Forberg AS, Larvik, Norway) for 300s. Thereafter, the blend was fed into a double shaft conditioner (BCTC 10, Bühler, Uzwil, Switzerland) before being subjected to extruder processing (Twin Screw Bühler BCTG 62 Extruder, Bühler, Uzwil, Switzerland) with die size of either 3 or 5 mm. The nine extruded concentrates were grouped into three groups according to processing parameters with respect to screen size of the hammer mill and die size of the extruder (Table 4). The concentrates were extruded under different extrusion conditions: 1) as such (with neither steam injection nor water cooling); 2) steam injection into section 4 of the extruder barrel; 3) water cooling for section 5 of the extruder barrel.

Table 4. Processing parameters and conditions for the nine extruded concentrates.

Processing Parameters[1] (mm)	Group 1: HM, 2; Die, 3			Group 2: HM, 2; Die, 5			Group 3: HM, 4; Die, 5		
Conditions for Concentrates[2] (1-9)	EAS (1)	ESI (2)	EWC (3)	EAS (4)	ESI (5)	EWC (6)	EAS (7)	ESI (8)	EWC (9)

[1] HM: Screen size of the hammer mill, Die: Die size of the extruder.

[2] EAS: extruded as such, ESI: extruded with steam injection into 4th section of extruder, EWC: extruded with water cooling for the 5th section of the extruder. Different from the abbreviations (EAS, ESI and EWC) used for the pilot concentrates, the abbreviations for the experimental concentrates were Eas, Esi and Ewc.

Production of the experimental concentrates

Physical property analyses of the nine extruded concentrates showed that the first three concentrates in Group I were close to the requirement for the experimental concentrates. Therefore, they were selected as a reference regarding processing parameters using 2 mm screen size of the hammer mill and 3 mm die size of the extruder to produce the three extruded concentrates used for the animal experiment in the second step of production. In addition, an adjustment on the ratio of barley to SBM was made to improve it from 65: 35 to 70: 30 for all four experimental concentrates. The control concentrate was produced using a hammer mill with 4 mm screen size and an expander with 5 mm die size (Kahl OE 23 Annular Gap Expander, Reinbek, Germany), expanded at 110 ^{o}C prior to being pelleted (Pellet Press, RPM 350.100, Munch-Edelsthal, Wuppertal, Germany).

Analysis of physical properties conducted upon the four experimental concentrates were performed as described later. Descriptions and expectations of the four experimental concentrates were as follows:

Concentrate Eas: High water stability and slow sinking pellets, expected to have high possibility of rumen escape (extruded as such).

Concentrate Esi: High water stability and floating pellets, expected to have low possibility of rumen escape (extruded with steam injection into the 4th section of the extruder).

Concentrate Ewc: High water stability and fast sinking pellets, expected to have intermediate possibility of rumen escape (extruded with water cooling for the 5th section of the extruder).

Control: Low water stability and fast sinking pellets, expected to have low possibility of rumen escape (concentrate processed by an expander).

3.2 Analyses of physical properties

Samples of each concentrate were taken directly after drying. The samples were subjected to physical property tests. The physical property tests were bulk density, pellet hardness, water stability and sinking velocity.

The bulk density was measured using a plastic cylinder with 1L of volume. The pellets were loose poured into the cylinder until creating a pile of pellets on the top. A scrape was used to remove the pile over the edge of the cylinder to form a flat surface. The weight of the filled cylinder was recorded on an electrical scale. Three replicate measurements were made per concentrate.

The hardness was measured by Olsen texture analyzer (H5KT-0650, RH 1 5DZ, England) using diametral compression equipped with a 100 Newton load cell and a PC-operated remote control. A cylindrical flat-ended probe, fitted on a load arm, moved vertically at a constant speed towards the plate until achieving 60% compression on the pellet. At this point, breaking strength was reported in Newtons. Strength (hardness) was recorded as an average of thirty pellets.

Sinking characteristics of pellets was performed using a 250 ml cylinder filled with rumen liquor, placed in an incubator to maintain a temperature at 39 ^{o}C. Within each concentrate, thirty pellets with similar sizes and diameters were chosen at random and introduced gently one by one. Sinking velocity was determined by timing the descent between two marks, 22 cm apart in the cylinder. A rumen liquor supply was stored at constant temperature 39 ^{o}C and the rumen liquor was renewed prior to the next test concentrate.

Water stability test was performed using the Ankom Daisy incubator (Ankom Technology Corp, NY, USA) and the following laboratory modified procedure. Around 6 liters of rumen liquor was withdrawn from 3-4 cows and divided into three jars. In each jar, three circular wire netting baskets (inner diameter: 55-58 mm; mesh size: 0.7 mm), containing 5 g pellets each, were incubated at 39 ^{o}C for two hours. The extent of disintegration in mesh filter balls was recorded by visual inspections right after incubation. Dry matter disappearance determined by oven drying at 103 ^{o}C for 24 h was used to calculate water stability index (WSI).

The visual inspection of water stability was conducted upon all concentrates (the pilot concentrates and the four experimental concentrates). Representative pictures of the first three extruded concentrates, EAS (1), ESI (2) and EWC (3), and of the experimental concentrates were used to illustrate the visual differences in terms of incubation time and the ratio of barley to SBM.

3.3 Animal experiment

3.3.1 Animals and feeding

Animal experiment was performed at metabolism unit, IHA. Standard animal experiment conditions were authorized by the Norwegian Animal Research Authority. Experimental animals were four multiparous lactating Norwegian Red cows in early lactation (64±10 days post-partum), weighing 611±43 kg, with milk yield at the start of experiment 34±6 kg day^{-1}, of which all four were equipped with rumen cannula (Bar Diamond, Parma Idaho, USA; inner diameter: 100 mm) and open T-shaped duodenal cannula (made out of PVC with inner diameter of 25 mm) in the proximal duodenum 50-60 cm distal to pylorus. Cows were housed in tie stalls with rubber mat and had *ab libitum* access to fresh water from individual water bowls.

Feeding of animals was comprised of silage and the concentrates. In addition, a Multi mineral mix (Pluss Storfe multitilskudd, Felleskjøpet, Agri, Lillestrøm, Norway) was spread over silage at each feeding to yield 200 g/d. There were two types of silages, blended in a TMR-mixer wagon (Kverneland Duo 1814). Silage 1 was second cut from Sørås, with DM content of 37.6% and CP and NDF contents of 112 and 510 g/kg DM, respectively, whereas silage 2 was first cut from Nedre Norderås, with DM of 19.9% and CP and NDF contents of 158 and 533 g/kg DM, respectively. Silage was fed *ad libitum*, defined as a minimum of refusals of 10% of daily feed, at 7:30, 15:30 and 21:00 h at a ratio of 0.4, 0.4 and 0.2 of expected daily intake, respectively. For concentrates, a fixed daily ration of 10 kg per cow of one out of the four concentrates was assigned. The daily concentrate ration was divided into three equal meals and offered at 7:00, 15:00 and 20:30 h.

3.3.2 *In sacco* experimental design

The *in sacco* procedure for measuring rumen degradation was as described in Norfor system (Volden et al., 2011) except that pellets were not ground. Approximately 2g of pellets from each concentrate was filled into nylon bags with a pore size of 36µm. Bags from each type of concentrates were equally distributed among the four cows and incubated in the rumen for 0, 4, 8, 24 and 48 h. After removal from the rumen, the bags were immediately rinsed in cold tap water and followed by washing procedure in a washing machine. The zero-hour bags did not experience incubation in the rumen but the subsequent procedures from washing. Washed bags were dried at 45 ^{o}C for 48 h, and then weighed, whereupon the residues of replicates were pooled within incubation time and animals prior to being milled using a Retsch Mixer mill (Retsch, Haan,

Germany) for 30 seconds at a frequency of 50 Hz. The residues were stored at room temperature in air-tight glass jars until the determination of N and starch.

3.3.3 *In vivo* experimental design

The experiment was conducted as a balanced 4 x 4 Latin square design to account for carryover effects, with four concentrates, four cows and four periods. Each period consisted of 21 days with 11 days for adaption and 10 days for sampling.

Marker infusion

A dual-marker technique for determination of digestibility was applied using Cr (Cr-EDTA) and Yb (Yb-acetate) as external markers for liquids and particles, respectively. At 12:00 on day 3, pulse doses of Cr-EDTA solution (3.0 kg) and Yb-acetate solution (3.0 kg) were poured into rumen through rumen cannula. Right after the pulse dose, continuous infusion of Yb-acetate (ca. 3 kg/d) and Cr-EDTA (ca. 3 kg/d) were given through a pipe connected to a bucket container, using a peristaltic pump to facilitate the infusion.

Sampling and sampling protocol

In each experimental period, representative samples of concentrates were collected and analyzed for DM, ash, fat, N, starch and NDF.

Recording of continuous rumen pH

From 15:00 day 17, rumen pH was logged every 10^{th} minute for 24 hours with pH-meters (WTW 3320, Weilheim, Germany) equipped with liquid tight electrodes attached to a stainless-steel sink. The electrodes were placed in a perforated rubber tube fitted to the cannula lid and hung in the rumen 10-15 cm above bottom of the ventral sac. Time spent blow a certain pH point was recorded by counting the pH values that were lower than the examined pH in a period of 24 h, multiplied by 10 min, expressed as h/d.

Sampling of rumen liquor

On day 17, rumen liquor for determination of volatile fatty acids (VFA), ammonia, rumen fermentation patterns, was withdrawn from ventral, medium and dorsal sac of the rumen, respectively, at each hour from 07:00 until 05:00, by a syringe fitted with a rumen sampler. Samples were transferred to 15 ml polystyrene tubes with 0.5 ml of formic acid (analytical grade),

after which the tubes were closed and turned upside down for sufficient blending. The remaining rumen liquor was subjected to pH measurement with a pH meter (described above).

Sampling of duodenal digesta

On day 12 (09:00, 15:00 and 22:00), day 13 (04:00, 12:00 and 18:00) and day 14 (01:00 and 13:00), duodenal digesta (500 g) were collected using plastic bags for digestibility determination. Thereafter, samples were transferred to collecting baskets prior to pH measurement. Each sampling was done with the same order among cows. After collection was completed, digesta was thawed and divided into eight ca. 888 ml aluminum trays along with stirring and immediately frozen to -21 ^{o}C until freeze-drying.

Quantitative collection and sampling of feces and urine

From 08:00 day 11 until 08:00 day 14 (72 hours), feces and urine were collected quantitatively and kept separately, both of which were collected in buckets at spontaneous excretion. Feces or urine not collected this way were kept separately and collected from steel trays kept under the metabolism boxes. All materials were registered and immediately transferred to collection buckets, which was changed every 8th hour and kept cold. Each urine collection bucket was prepared with 0.5 L sulfuric acid (pH was checked and should be below 5). Every 24th hour, collected feces within cow were blended with a concrete blender for 3 minutes, after which 10 % was transferred to a collection sample and kept frozen. Urine from 24 hours collection was manually mixed and like feces, 10 % of urine were transferred to a collection container and kept frozen at -21˚C.

3.4 Chemical analysis of samples

The DM content (103 ^{o}C) and ash were determined using ISO 5984 method (550 ^{o}C for a minimum of 4 h). Nitrogen content was analyzed according to Kjeldahl-N Method 2001.11 (AOAC, 2002). CP was estimated as N × 6.25. Crude fat was analyzed by Accelerated Solvent Extraction (ASE) method. Neutral detergent fiber (NDF) were determined with Van Soest et al. (1991), using the Ankom 220 fiber analyzer (ANKOM Inc., Fairport, NY, USA). Content of starch in concentrates and residues after rumen incubation was determined as glucose after hydrolysis by α-amylase and amylo-glucosidase (McCleary et al., 1994). Analysis of chromium and ytterbium were carried out as described by MP-AES method (Agilent 4200 MP-AES, Agilent Technology, Melbourne, Australia). Volatile fatty acids were analyzed by gas chromatography (TRACE 1300 Gas Chromatograph equipped with Stabilwax-DA column 30 m, 0.25 mm i.d., 0.25 µm; Thermo

Fischer Scientific S.p.A., Milan, Italy). Ammonia nitrogen (NH_3-N) was analyzed using Method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) with a modification that block digestion was not carried out.

3.5 Calculations and statistical analysis

Rumen degradation parameters of starch and protein were processed using NLIN procedure of SAS (SAS, 9.4), based on the model (Ørskov and McDonald, 1979): $Y(t) = A + B(1 - e^{-Ct})$, where Y is degradation after t hours, A is the material immediately degraded (%), B is the material degraded over time (%), C is the fractional degradation rate of B (h^{-1}) and t is time. Effective degradability values of DM, starch and protein were estimated using the equation: $ED = A + [(B \times C)/(C + K)]$, where A, B and C are described above and K is the fractional rate of passage assumed to be 0.08/h (Madsen et al., 1995).

Statistical analysis was performed using SAS (SAS 9.4). The analysis of variance (GLM procedure) was used to test the effect of treatment on EDMD, ESD and EPD within concentrates, with treatment as main effect and animal as random effect. The Proc MIXED procedure was used to test the effect of treatment on VFA and pH with repeated measurements, using the default variance component as covariance structure. The least square means (LSM) was reported. Treatment effects were separated using the PDIFF statement. 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 the concentrates

Physical property analyses of the pilot concentrates

In Table 5, bulk densities of concentrates EWC (concentrates 3, 6 & 9) were greater than that of concentrates Eas (concentrates 1, 4 & 7) and ESI (concentrates 2, 5 & 8) (P=0.01). The last group showed higher bulk densities than the first (P=0.04) and the second group (P=0.03). The different conditions applied during extruder processing gave considerable variation on bulk density among concentrates one to three using 2 mm screen size of the hammer mill and 3 mm die size of the extruder, and among concentrates four to six using 2 mm screen size of the hammer mill and 5 mm die size of the extruder (Table 5). Bulk density of concentrates seven to nine using 4 mm screen size of the hammer mill and 5 mm die size of the extruder were not markedly different from each other and were in general high (Table 5).

With respect to hardness and sinking velocity, no significant differences were found neither among conditions nor among groups (Table 5). The variation of hardness was from 40 Newton in concentrate 2 to 87 Newton in concentrate 6. The numerically low-density concentrates (concentrates 1, 2, 4 & 5) resulted in pellets floating (sinking velocity=0), whereas the high densities (concentrates 3, 6-9) caused pellets sinking with varying velocities.

Water stability index (WSI) of the concentrates differed among conditions (P=0.01) and among groups (P<.01) (Table 5). The first two groups showed higher WSI than the last group. In addition, concentrates ESI (concentrates 2, 5 & 8) were more water stable than the others.

Table 5. Physical quality of concentrates based on the given processing parameters and conditions.

Processing Parameters[1] (mm)	Group I: HM, 2; Die, 3			Group II: HM, 2; Die, 5			Group III: HM, 4; Die, 5				*P*-values	
Conditions for concentrates[2] (1-9)	EAS (1)	ESI (2)	EWC (3)	EAS (4)	ESI (5)	EWC (6)	EAS (7)	ESI (8)	EWC (9)	SEM	Cond.[4]	Group[5]
Bulk Density (g/L)	513	452	596	513	439	588	600	570	611	17.91	0.03	0.06
Hardness (Newton)	47	40	73	65	48	87	48	51	57	7.92	0.09	0.25
Sinking velocity (sec/m)	0	0	30	0	0	17	24	15	11	6.73	0.37	0.56
WSI[3] (%) (2h)	65	72	63	66	72	63	42	51	46	1.13	0.01	<.01

[1] HM: Screen size of the hammer mill, Die: die size of the extruder.

[2] EAS: extruded as such, ESI: extruded with steam injection into 4th section of extruder, EWC: extruded with water cooling for the 5th section of the extruder.

[3] Water stability indexes for incubation of 2 hours.

[4] Comparison between conditions.

[5] Comparison between groups.

Physical property analyses of the experimental concentrates

Physical quality of the four concentrates used in the animal experiment is shown in Table 6. Of the extruded concentrates, temperature ranged from 79 ºC for concentrate Ewc to 125 ºC for concentrate Esi. This yielded graded bulk density from 650 to 410 g/L, hardness from 100 to 48 Newton and WSI at incubation of 2h from 75% to 59% ($P<0.05$). The WSI did not differ among the extruded concentrates at incubation of 1 h. Concentrate Esi was floating, concentrate Eas slow sinking and concentrate Ewc fast sinking, with sinking velocity 0, 11 and 29 sec/m, respectively. Compared with the extruded concentrates, the expander treated concentrate control showed higher bulk density, hardness, sinking speed and lower WSI at incubation of both 1 and 2 h.

Table 6. Temperature and physical properties of the experimental concentrates.

Experimental Concentrates[1]	Temperature[2] (°C)	Bulk density (g/l)	Hardness (Newton)	Sinking Velocity (sec/m)	Sinking characters[3]	WSI (%) 2h[4]	WSI (%) 1h[5]
Eas	106	545[b]	72[c]	29[c]	SS	70[b]	83[a]
Esi	125	410[c]	48[d]	0	F	59[c]	83[a]
Ewc	79	650[a]	100[b]	11[b]	FS	75[a]	87[a]
Control	110	670[a]	206[a]	7[a]	FS	50[d]	62[b]

[1] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Temperature measured in 5th section of the extruder for the three extruded concentrates (hereafter referred to as temperature); Temperature measured in die of the expander for the control (hereafter referred to as $temperature_p$).

[3] Sinking characters were classified as floating (F), slow sinking (SS) and fast sinking (FS).

[4] Water stability indexes for incubation of 2 hours.

[5] Water stability indexes for incubation of 1 hour.

[6] Means followed by different letters indicate statistical difference as $P<0.05$.

Visual inspection of water stability

Visual inspection of water stability is shown in Figure 12. At incubation of 2h, pellets of concentrate Eas and Ewc appeared to remain intact when the proportion of barley increased, but this increase failed to keep visible pellets of concentrate ESI. At the same ratio of barley vs SBM (70: 30), shorter incubation time (1h) resulted in more pellets with complete structure and less residues on the basket.

* The first row showed the first three extruded concentrates (Barley: SBM, 65: 35) processed in the 1st step, whereas the second and the third rows showed the experimental concentrates (Barley: SBM, 70: 30) processed in the 2nd step. The difference between the first two rows and the last row was incubation time. The same column indicated the same condition during extruder processing.

Figure 12. Visual inspection of the concentrates.

Figure 13 shows that under the same extruder processing condition, the increase of barley content resulted in the increase of WSI from 65% (EAS) to 75% (Eas) and from 63% (EWC) to 75% (Ewc) but the decrease of that from 72% (ESI) to 59% (Esi). In addition, the group with higher proportion of barley showed comparatively lower standard deviation.

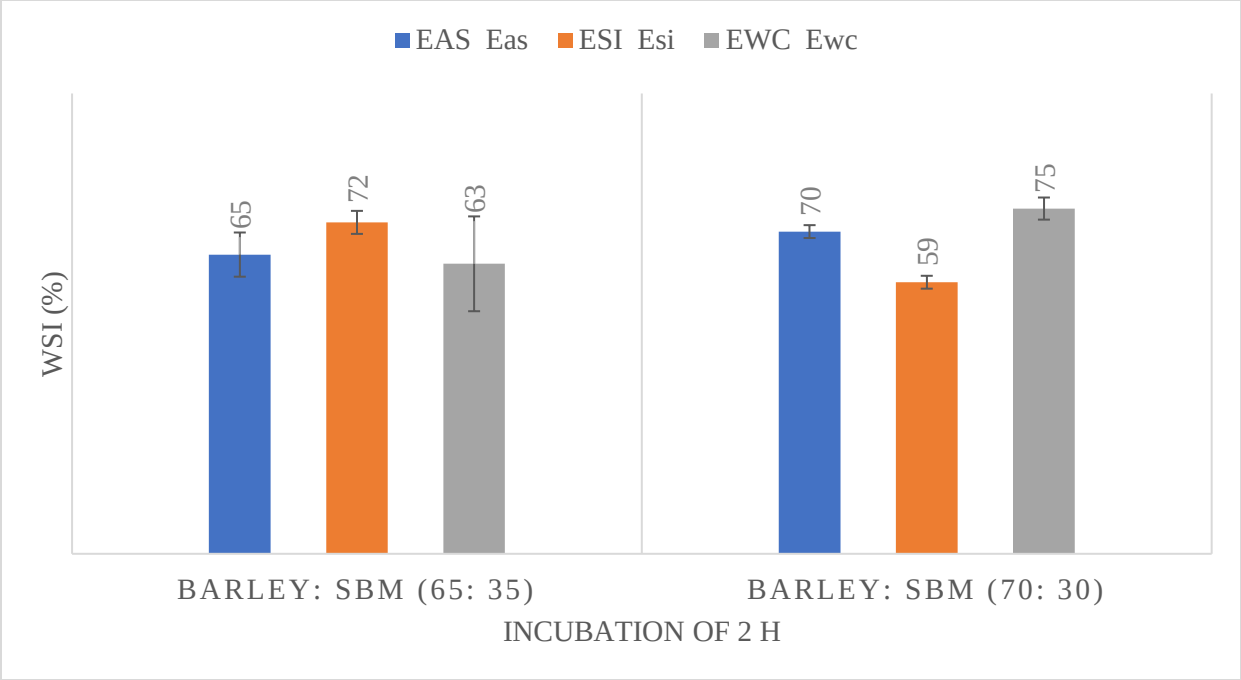

Figure 13. Water stability index (WSI) for the first three extruded concentrates (Barley: SBM, 65: 35), and for the three extruded concentrates (Barley: SBM, 70: 30) used in the animal experiment, at incubation of 2h.

4.2 Chemical composition of the experimental concentrates

The chemical analyses for concentrates and silage are presented in Table 7. The content of dry matter varied between 866 and 902 g/kg for the experimental concentrates. Content of starch was lowest in the concentrate Esi (406 g/kg) and was highest in the control (428 g/kg). The content of protein ranged from 221 g/kg in the concentrate Eas to 233 g/kg in the concentrate Esi. The content of NDF was low for the concentrate Eas (175 g/kg) and control (174 g/kg) and was high for the concentrate Esi (192 g/kg) and Ewc (199 g/kg). Extrusion cooking seems to decrease fat content as compared to the control.

Table 7. Chemical composition of the experimental concentrates and silage.

Concentrates[1]	DM, g/kg	Chemical Composition, g/kg DM					
		Starch	CP	NDF	Ash	Fat	WSC[3]
Eas	886	422	221	175	32	2.5	-
Esi	898	406	233	192	35	3.6	-
Ewc	866	425	225	199	32	4.2	-
Control	902	428	232	174	33	9.8	-
Favør 80[2]	888	348	194	214	62	54	-
Silage	264	-	112	510	56	34	113

[1] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Commercial concentrate.

[3] Water soluble carbohydrates.

4.3 *In sacco* measurements

In sacco ruminal degradation of dry matter

Ruminal DM degradation as a function of time and ruminal degradation characteristics of DM are presented in Figure 14 and Table 8, respectively. In Figure 14, the DM degradation for the control was highest at incubation of 0 and 4 h. With time, the difference of dry matter degradation between the control and the extruded concentrates decreased gradually.

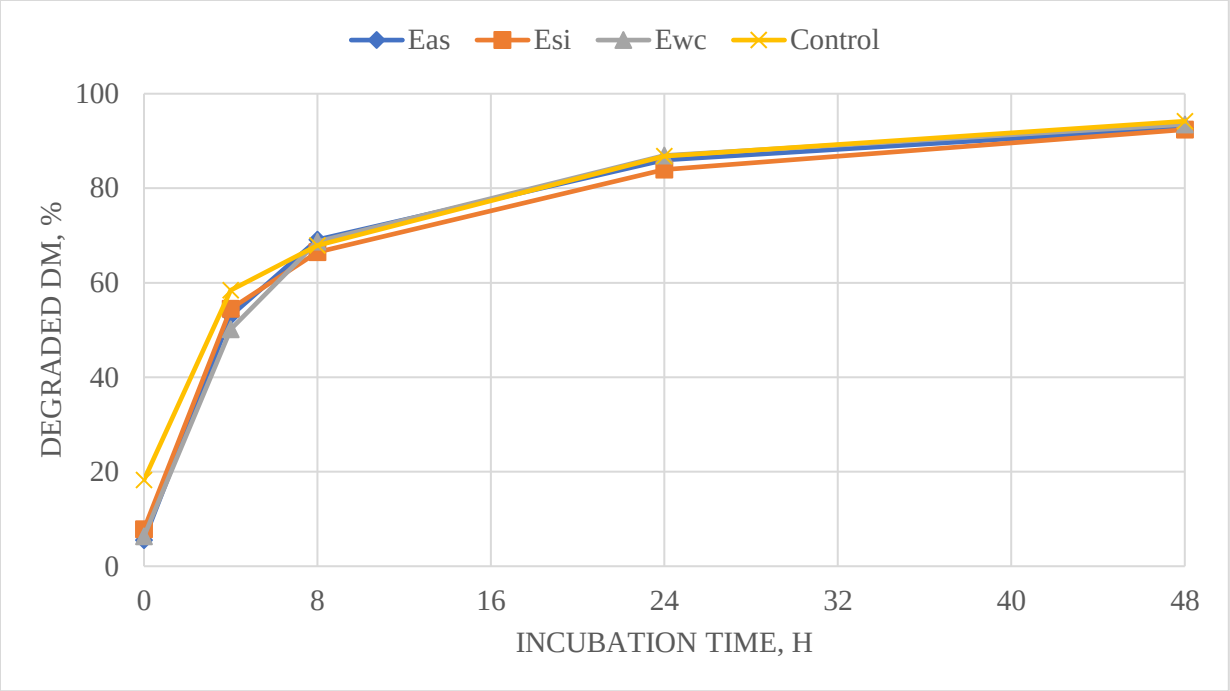

Figure 14. *In sacco* degradation profile of dry matter in the concentrates Eas, Esi, Ewc and control.

As compared to the control, extrusion significantly decreased A-fraction (P<.01) and EDMD (P=0.03) but increased B-fraction (P<.01) (Table 8). Compared with the control, the concentrate Eas showed lowest A-fraction, the concentrate Ewc showed highest B-faction and all extruded concentrate resulted in higher EDMD which were statistically similar.

Table 8. In sacco rumen degradation characteristics of dry matter.

	Concentrates[2]					*P*-values	
Items[1]	Eas	Esi	Ewc	Control	SEM	Concentrate	Contrast[3]
A	5.8^{c}	8.8^{b}	6.7^{c}	19.6^{a}	0.29	<.01	<.01
B	83.8^{a}	79.5^{b}	84.4^{a}	71.4^{c}	0.88	<.01	<.01
C	20.2	18.9	17.7	16.4	0.94	0.09	0.25
EDMD	64.9^{b}	64.3^{b}	64.2^{b}	67.5^{a}	0.71	0.03	0.14

[1] A: DM immediately degraded (solubilized at incubation time 0), B: DM not soluble but potentially degraded, C: Fractional rate of degradation of B (h^{-1}), EDMD: Effective DM degradability calculated using a fractional rate of passage (K) of 0.08h.

[2] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[3] The extruded concentrates vs the control

[4] Means followed by different letters indicate statistical difference among the concentrates as $P<0.05$.

In sacco ruminal degradation of starch

Ruminal starch degradation as a function of time is shown in Figure 15. The degradation of starch showed the ranking at incubation of 0 h from high to low: the control, Esi, Eas and Ewc, as well as at 4 h. After 4 h, the difference between the concentrates decreased.

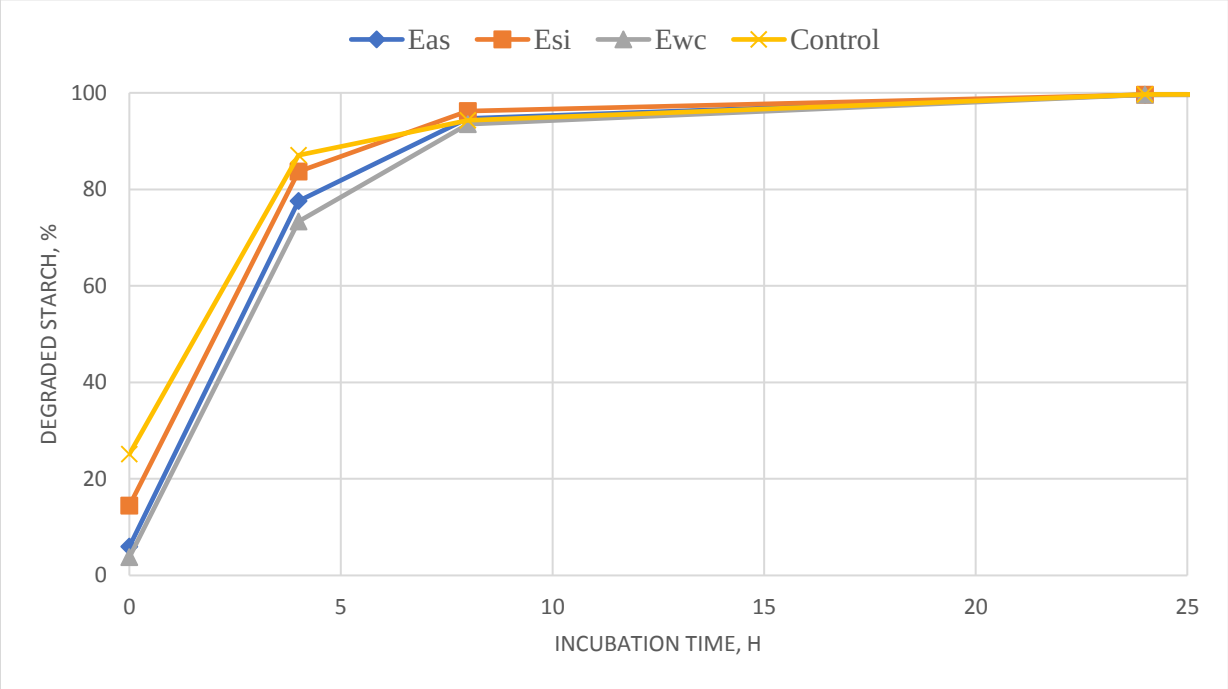

Figure 15. *In sacco* degradation profile of starch in the concentrates Eas, Esi, Ewc and control.

Ruminal degradation characteristics of starch are shown in Table 9. The extruded concentrates resulted in the decreased A-fraction and the increased B-fraction in comparison with the control ($P<.01$). Comparing the concentrates, concentrate Ewc showed lowest A-fraction ($P<.01$), highest *B*-fraction ($P<.01$) and significantly higher ESD than the control.

Table 9. *In sacco* rumen degradation characteristics of starch.

	Concentrates[2]					*P*-values	
Items[1]	Eas	Esi	Ewc	Control	SEM	Concentrate	Contrast[4]
A	5.5[c]	14.5[b]	3.4[d]	25.2[a]	0.24	<.01	<.01
B	93.8[b]	85.1[c]	96.1[a]	73.7[d]	0.42	<.01	<.01
C	43.7	46.0	36.5	44.2	3.24	0.24	0.77
ESD	83.1[b]	86.1[ab]	81.0[b]	87.6[a]	1.36	0.03	0.15

[1] A: Starch immediately degraded (solubilized at incubation time 0), B: Starch not soluble but potentially degraded, C: Fractional rate of degradation of B (h^{-1}), ESD: Effective starch degradability calculated using a fractional rate of passage (K) of 0.08h.

[2] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[3] The extruded concentrates vs the control.

[4] Means followed by different letters indicate statistical difference among the concentrates as $P<0.05$.

In sacco ruminal degradation of protein

Ruminal protein degradation as a function of time is shown in Figure 16. Except incubation time of 4 h, the protein degradation for concentrate Esi was lowest throughout incubation time from 0 to 48 h. On the contrary, concentrate Ewc exhibited highest protein degradation at most of incubation times. In addition, a negative value occurred to concentrate Esi at incubation of 0 h.

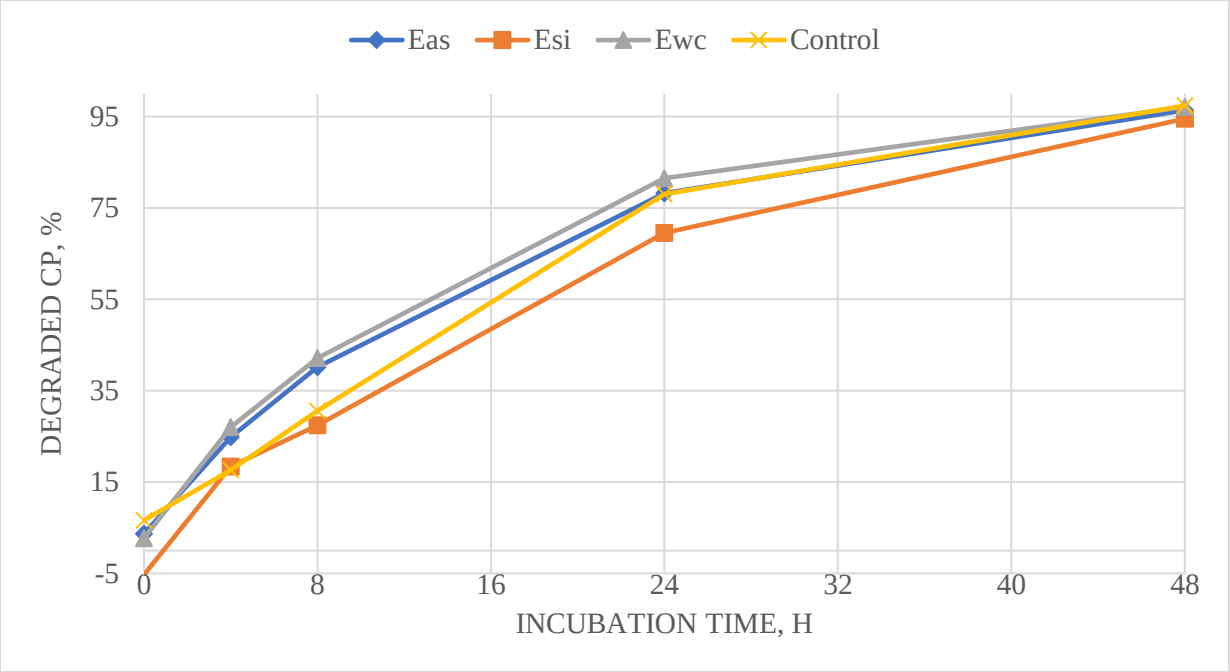

Figure 16. *In sacco* degradation profile of protein in the concentrates Eas, Esi, Ewc and control.

Ruminal degradation characteristics of protein are presented in Table 10. Feeding concentrates Eas and Ewc resulted in higher C-fraction and EPD than feeding the control (P<.01). The A-fraction was increased the most by feeding concentrate Eas, whereas the B-fraction and EPD were decreased by feeding concentrate Esi as compared to the control (P<0.05).

Table 10. *In sacco* rumen degradation characteristics of protein.

	Concentrates[2]					*P*-values	
Items[1]	Eas	Esi	Ewc	Control	SEM	Concentrate	Contrast[3]
A	3.6^{a}	0.0^{b}	2.7^{ab}	1.4^{b}	0.6	0.01	0.45
B	96.4^{b}	100^{a}	97.4^{b}	98.6^{ab}	0.6	0.01	0.45
C	6.2^{b}	5.0^{d}	6.9^{a}	5.6^{c}	0.15	<.01	<.01
EPD	45.6^{b}	37.9^{d}	47.5^{a}	41.7^{c}	0.41	<.01	<.01

[1] A: Protein immediately degraded (solubilized at incubation time 0), B: Protein not soluble but potentially degraded, C: Fractional rate of degradation of B (h^{-1}), EPD: Effective protein degradability calculated using a fractional rate of passage (K) of 0.08h.

[2] Eas: extruded as such; Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[3] The extruded concentrates vs the control.

[4] Means followed by different letters indicate statistical difference among the concentrates as $P < 0.05$.

4.4 Correlations

The effects of temperature on physical qualities of the experimental concentrates and the pilot concentrates were investigated (Figure 17). Hardness (H), bulk density (BD) and water stability index (WSI) of the experimental concentrates were all negatively correlated to temperature, showing strong relationships (A). However, an opposite trend regarding WSI with temperature were observed on all pilot concentrates (B, C & D). The values of R^2 were high for all correlations in the figure except that the correlation of WSI for the pilot concentrates (7, 8 & 9) with temperature was very close to 0.

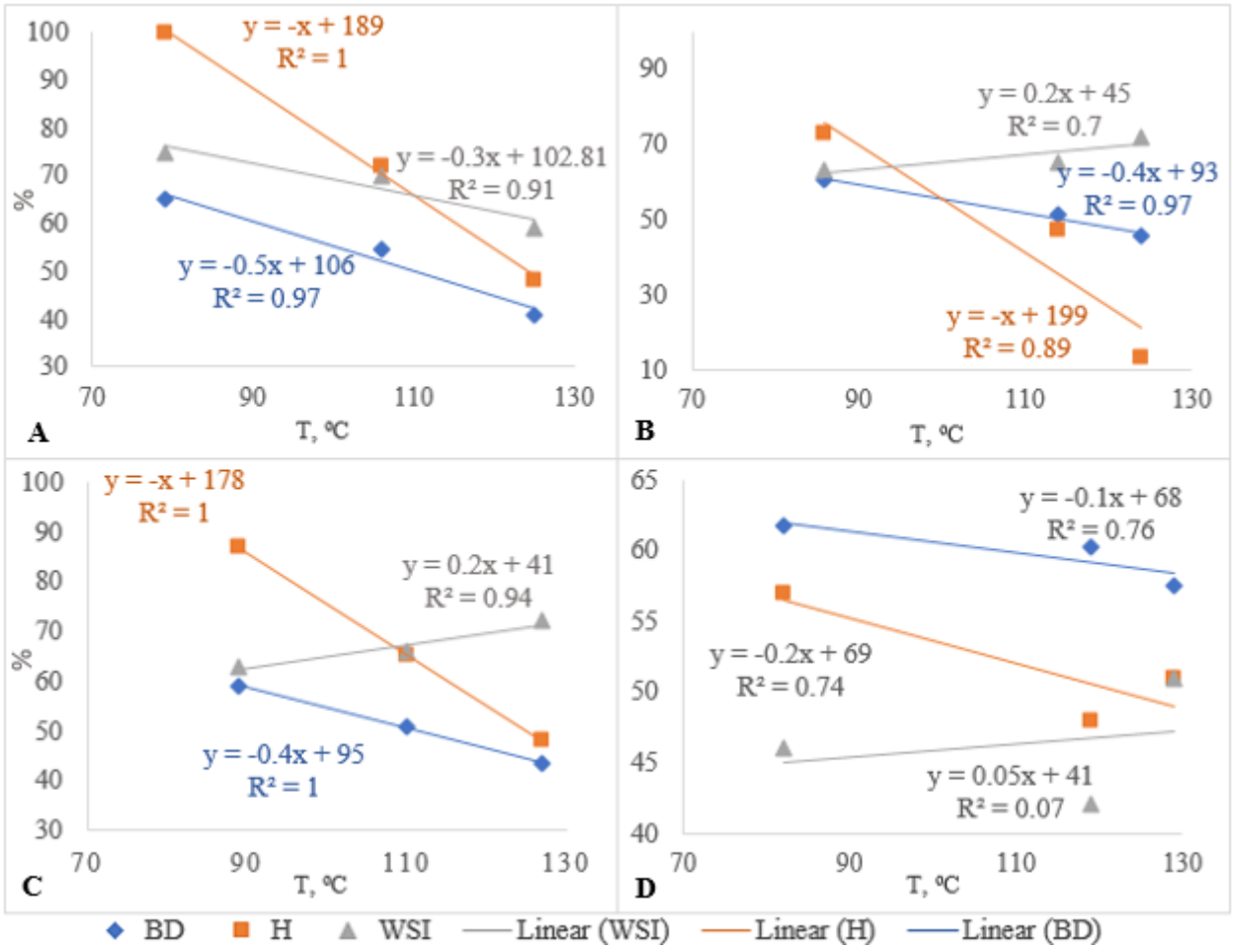

Figure 17. Effects of temperature (T, °C) on bulk density (BD), hardness (H) and water stability index (WSI) of the extruder treated experimental concentrates (A) and of the pilot concentrates 1, 2 & 3 (B); 4, 5 & 6 (C); 7, 8 & 9 (D).

The effects of temperature on ruminal degradation characteristics are shown in Figure 18. For the extruder treated experimental concentrates (Figure 18a), effective starch degradability (ESD) was positively related to temperature (R^2=0.96), whereas effective protein degradability (EPD) was negatively correlated to temperature (R^2=0.82). When the control was added (Figure 18b), R^2 for ESD decreased from 0.96 to 0.65 but for EPD the coefficient was almost not affected. There was no linear relationship between effective dry matter degradability (EDMD) and temperature regardless of the control added or not in the curve.

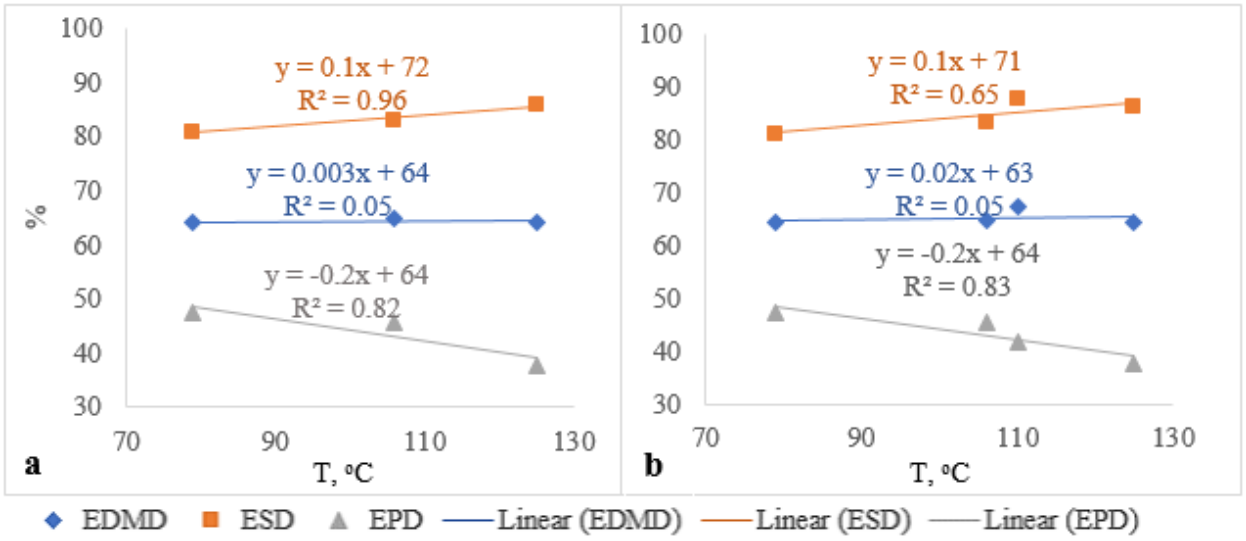

Figure 18. Effects of temperature (T, °C) on effective degradability of dry matter (EDMD), protein (EPD) and starch (ESD) of the extruder treated experimental concentrates (a) and of the four experimental concentrates (b).

In Figure 19, effective starch degradability (ESD) showed a downhill correlation of nearly 1 (R^2=0.99) with water stability index (WSI). Comparing ESD and with EDMD, EDMD was correlated with WSI in a negative model as ESD, but the correlation coefficient (R^2 = 0.6) was not as strong as that of ESD. On the contrary, effective protein degradability was positively affected by WSI, showing an uphill trendline. However, the correlation coefficient was moderate (R^2=0.58).

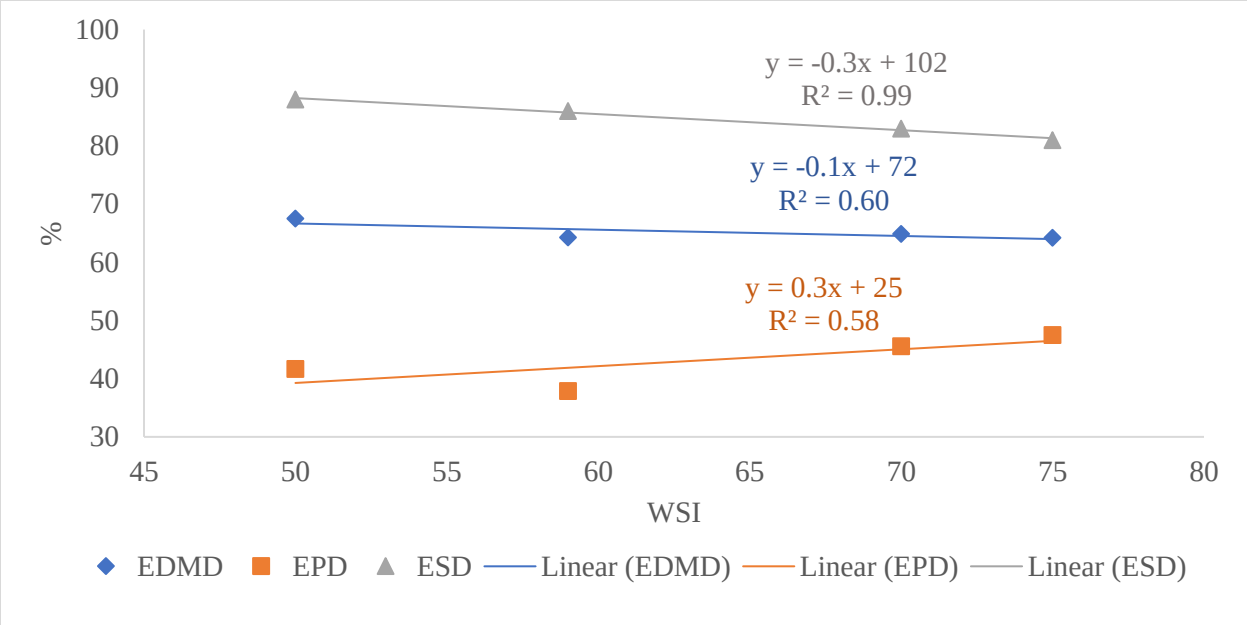

Figure 19. Effects of water stability index (WSI) at 2 h of incubation on effective degradability of dry matter (EDMD), protein (EPD) and starch (ESD), respectively, of the four experimental concentrates.

4.5 *In vivo* measurements

4.5.1 Ruminal 24-h pH and postprandial rumen fermentation

Mean daily ruminal pH was significantly lower for the extruded concentrates than for the control, whereas no differences were observed among the extruded concentrates (Table 11). Ruminal pH pattern over a 24-h period is shown in Figure 20.

Table 11. Ruminal pH (24 h), postprandial pH, concentrations of VFA and ammonia in the rumen.

	Experimental concentrates[1]					*P*-Values		
Items	Eas	Esi	Ewc	Control	SEM	Cons[2]	Time	Time×Cons
pH (24 h)	6.04[b]	6.02[b]	6.05[b]	6.12[a]	0.05	<.01	<.01	0.52
1-8 h post feeding								
Rumen:								
pH	6.30[b]	6.31[ab]	6.32[ab]	6.36[a]	0.03	0.17	<.01	0.80
Total VFA, mmol/L	95.2	92.2	92.1	91.7	1.79	0.51	<.01	0.90
Acetate, % of VFA	65.7	67.2	65.6	65.2	0.46	0.78	<.01	0.91
Propionate, % of VFA	17.8[ab]	18.7[a]	18.0[ab]	17.7[b]	0.52	0.20	<.01	0.94
Butyrate, % of VFA	13.3[ab]	13.1[b]	13.3[ab]	14.0[a]	0.60	0.18	<.01	0.91
Isobutyrate, % of VFA	0.72[b]	0.64[a]	0.69[b]	0.72[b]	0.04	0.01	<.01	0.95
Valerate, % of VFA	1.35	1.36	1.30	1.36	0.05	0.70	<.01	0.82
Isovalerate, % of VFA	1.18	1.08	1.14	1.10	0.12	0.68	<.01	0.68
Acetate: Propionate	3.75	3.53	3.72	3.75	0.13	0.45	<.01	0.88
Ammonia, mg/L	74.8	77.2	81.4	83.7	12.0	0.88	<.01	0.95

[1] Eas: extruded as such; Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Concentrate.

[3] Means followed by different letters indicate statistical difference as $P<0.05$.

For the four concentrates, fluctuations of ruminal pH over a 24-h period were similar (Figure 20). Ruminal pH dropped immediately after each feeding and subsequently started to increase until approaching next feeding. Nadir occurred for all concentrates after evening feeding and concentrate Ewc took more time to reach its nadir as compared to others. In addition, the extruded concentrates exhibited lower nadir than the control.

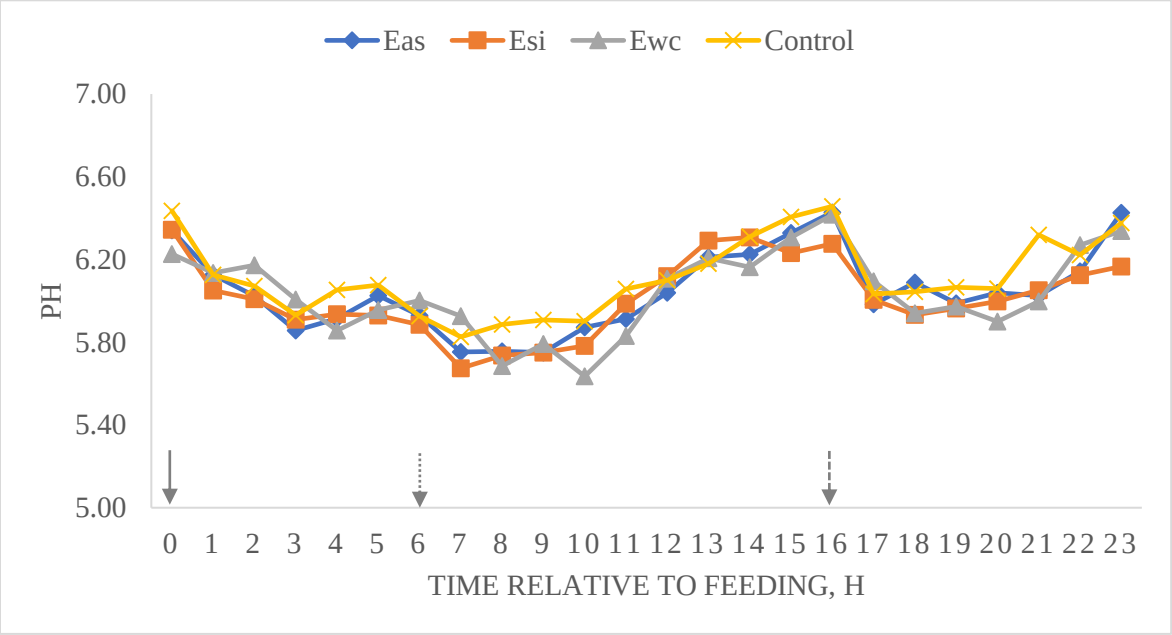

Figure 20. Ruminal pH for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over a 24-h period. Arrows show feeding times at 1500 (solid arrow), 2100 (dashed arrow) and 0700 (dotted arrow), respectively.

Time spent below examined pH for the concentrates is shown in Table 12. No differences of duration below any pH investigated were observed among the concentrates, except that the cows fed concentrates Eas (P=0.1) and Esi (P=0.08) tended to spend more time below pH 5.8 as compared to the control concentrate. Furthermore, time spent for the extruded concentrates below all pH points investigated were numerically higher than that for the control.

Table 12. Time spent below a certain pH point over a 24-h period.

Items	Concentrates[1]				SEM	*P*-Values	
	Eas	Esi	Ewc	Control		Concentrate	Contrast[2]
pH < 6.4, h/d	21.6	22.2	22.4	21.2	1.25	0.91	0.60
pH < 6.2, h/d	17.8	18.5	16.8	15.0	1.79	0.57	0.23
pH < 6.0, h/d	10.3	10.7	9.8	7.2	2.56	0.78	0.33
pH < 5.8, h/d	4.5	4.8	4.3	1.9	0.96	0.22	0.19
pH < 5.6, h/d	1.5	1.7	1.2	0.3	0.65	0.44	0.14

[1] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] The extruded concentrates vs the control.

Patterns of postprandial pH, VFA and acetate to propionate ratio

Postprandial pH decreased when cows were fed concentrate Eas compared to the control (Table 11). No significant differences among the concentrates were observed with respect to mean concentrations of VFA (Table 11). Feeding concentrate Esi to cows resulted in significantly higher concentration of propionate but significantly lower concentrations of butyrate and isobutyrate in comparison with feeding the control (Table 11). The postprandial pH patterns for the concentrates were quite similar (Figure 21). On all concentrates, a decrease in pH was observed after feeding, accompanied with a steady increase in pH 2-3 hours post feeding (Figure 21).

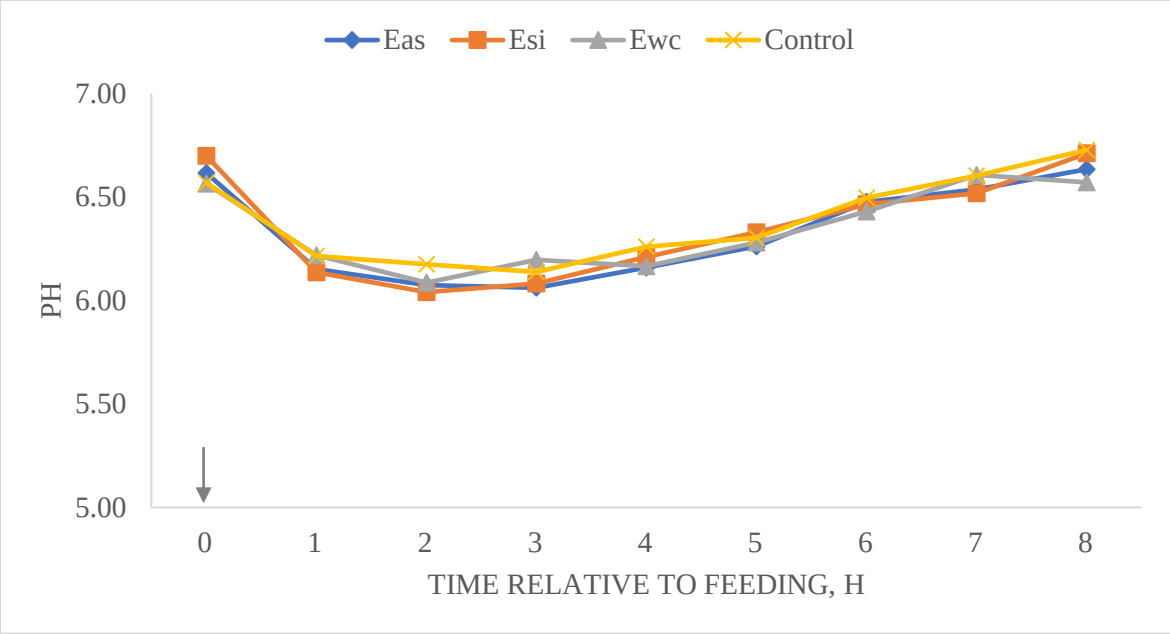

Figure 21. Postprandial (1-8 h) pH in rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively. Arrow shows feeding time at 0700.

Being opposite to the patterns of the postprandial pH, concentrations of VFA started out to increase after feeding, followed by trends of going down 2-3 hours post feeding (Figure 22). Cows fed concentrate Eas showed highest peak 2 h post feeding.

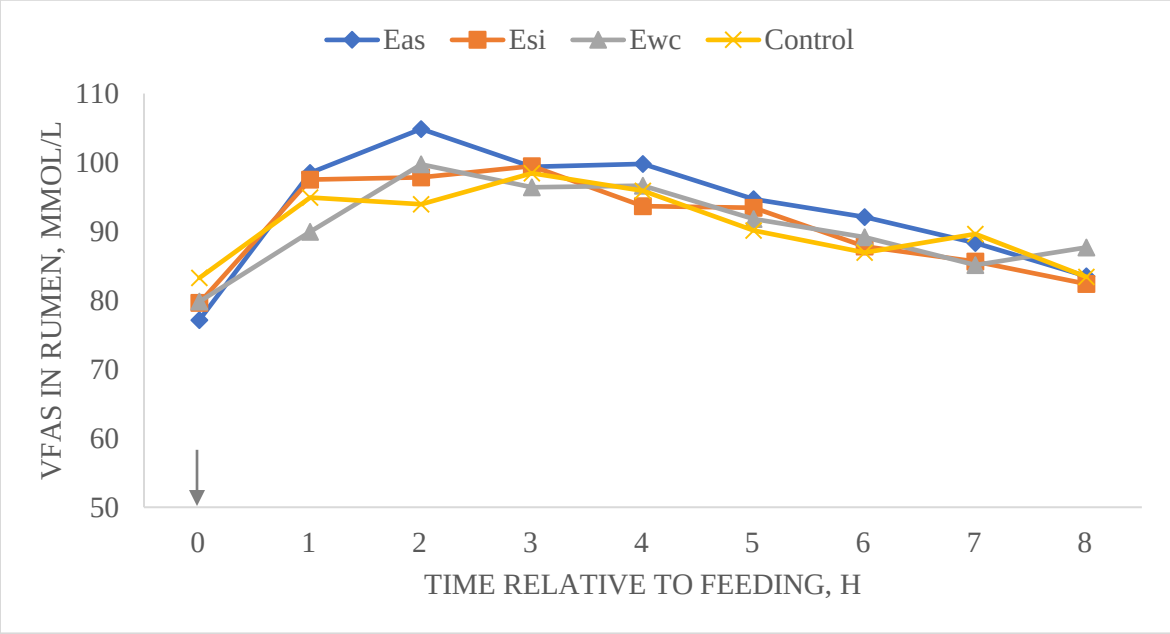

Figure 22. Postprandial (1-8 h) concentration of VFA in the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively. Arrow shows feeding time at 0700.

The patterns of acetate to propionate ratio (Figure 23) were similar to that of ruminal pH (Figure 21) after feeding. Lowest ratio of acetate to propionate was observed 2 h post feeding when cows were fed concentrate Esi. During the increase of the ratio after reaching the nadir, feeding concentrate Ewc caused a drastic fluctuation.

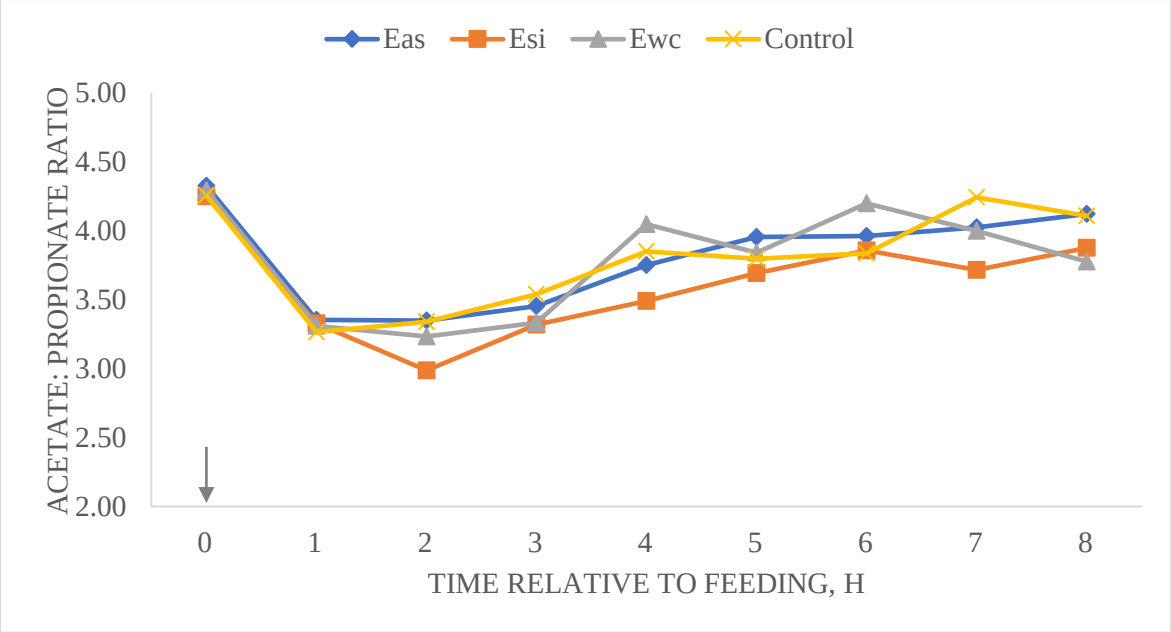

Figure 23. Postprandial (1-8 h) ratio of acetate to propionate in rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively. Arrow shows feeding time at 0700.

4.5.2 Postprandial fermentation in dorsal sac of the rumen

In dorsal sac of the rumen, postprandial pH was significantly lower when feeding concentrates Eas and Esi than feeding the control (Table 13). Total concentrations of VFA in dorsal sac were similar for the four concentrates. The molar proportions of both propionate and isobutyrate were higher when feeding concentrate Esi than the others ($P<0.05$). Compared with the control, the extruded concentrates resulted in significantly higher molar proportion of butyrate. The significant increase of propionate when feeding concentrate Esi did not cause a decrease of acetate to propionate ratio ($P>0.05$) (Table 13).

Table 13. Postprandial pH, concentrations of VFA and ammonia in dorsal sac of the rumen.

	Experimental concentrates[1]					*P*-Values		
Items	Eas	Esi	Ewc	Control	SEM	Cons[2]	Time	Time×Cons
Dorsal sac:								
pH	6.31[b]	6.30[b]	6.38[a]	6.42[a]	0.04	0.01	<.01	0.47
Total VFA, mmol/L	91.4	89.0	87.4	87.0	2.37	0.55	<.01	0.57
Acetate, % of VFA	65.8	65.2	65.7	65.3	0.48	0.69	<.01	0.68
Propionate, % of VFA	17.6[b]	18.8[a]	17.9[b]	17.6[b]	0.54	0.05	<.01	0.73
Butyrate, % of VFA	13.3[b]	12.9[b]	13.2[b]	13.9[a]	0.59	0.06	<.01	0.99
Isobutyrate, % of VFA	0.71[b]	0.64[a]	0.70[b]	0.70[b]	0.03	0.01	<.01	0.86
Valerate, % of VFA	1.35	1.35	1.30	1.33	0.04	0.73	<.01	0.88
Isovalerate, % of VFA	1.17	1.08	1.15	1.08	0.12	0.65	<.01	0.64
Acetate: Propionate	3.81	3.51	3.76	3.77	0.14	0.19	<.01	0.64
Ammonia, mg/L	77.6	82.9	84.3	88.5	11.7	0.86	<.01	0.88

[1] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Concentrate.

[3] Means followed by different letters indicate statistical difference as $P<0.05$.

Patterns of pH, VFA and acetate to propionate ratio in dorsal sac of the rumen

The pH patterns in dorsal sac (Figure 24) were similar to that of ruminal pH after feeding. In Figure 24, the decreases of pH for concentrates Eas and Esi were more rapidly than that of the other two concentrates 1 h post feeding. Cows fed the control took longer time to reach the nadir as compared to feeding the other concentrates.

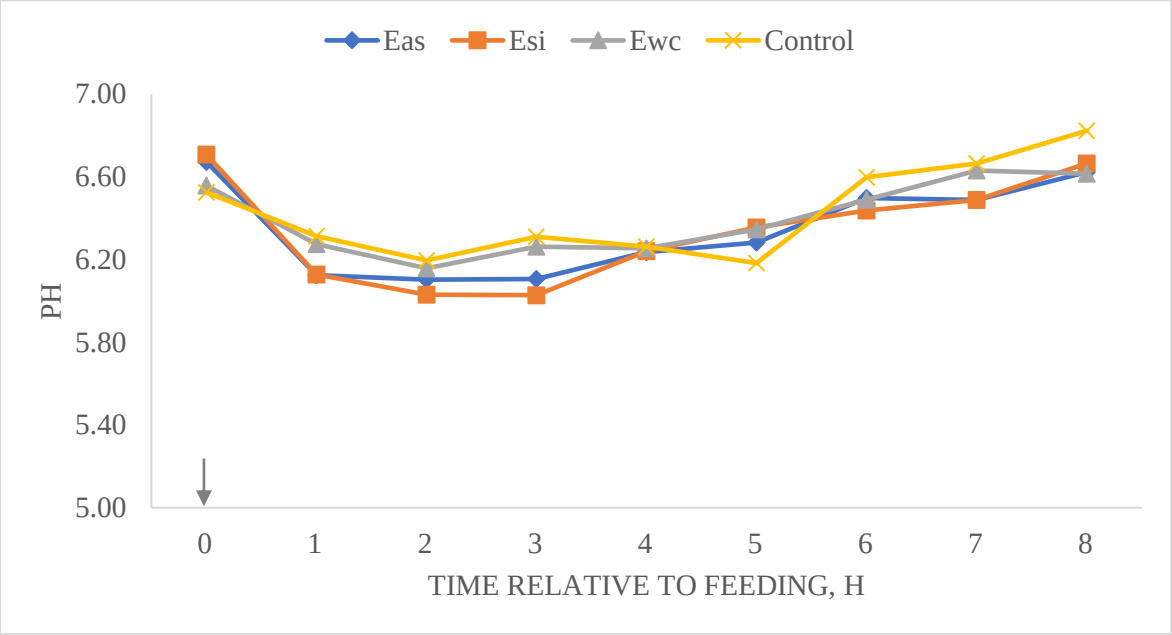

Figure 24. Postprandial pH in dorsal sac of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

Concentrations of VFA started out to increase after feeding, except that the control experienced a decline before starting to arise, with feeding concentrate Esi leading to the highest peak compared to feeding the other concentrates (Figure 25). A large fluctuation when feeding the control was observed 2 h before the next feeding.

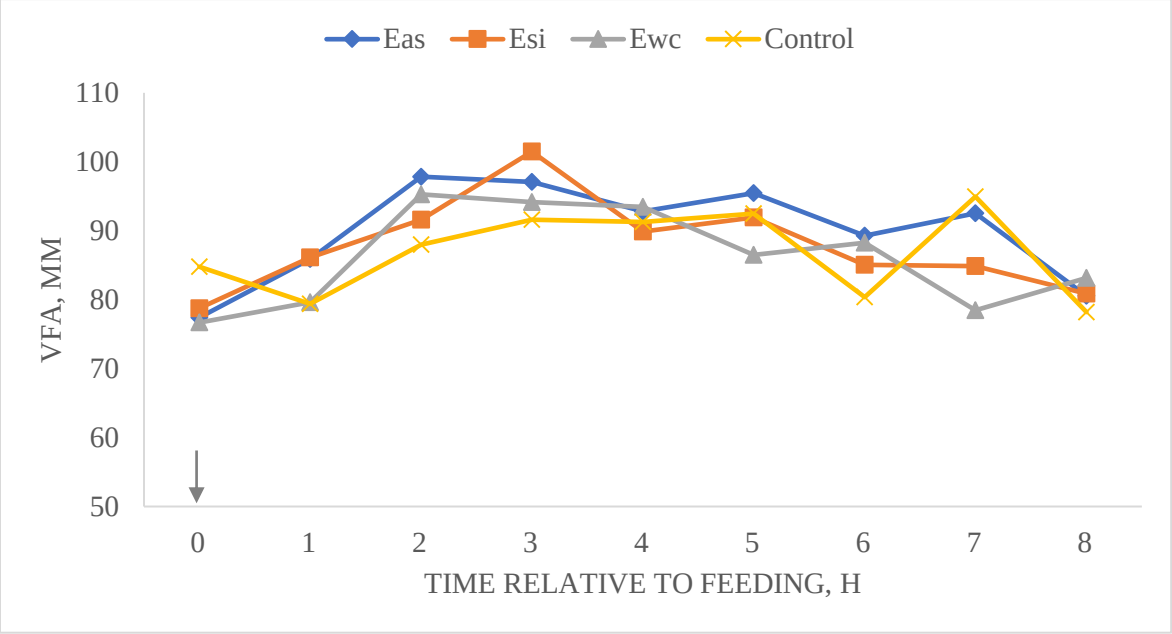

Figure 25. Postprandial total concentration of volatile fatty acids (VFA) in dorsal sac of rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

In Figure 26, the ratio of acetate to propionate for all concentrates showed a trend of declining after feeding, followed by an increase 1-3 h post feeding. The lowest ratio was observed feeding concentrate Esi, whereas the highest ratio occurred as a result of a sharp increase when feeding concentrate Ewc.

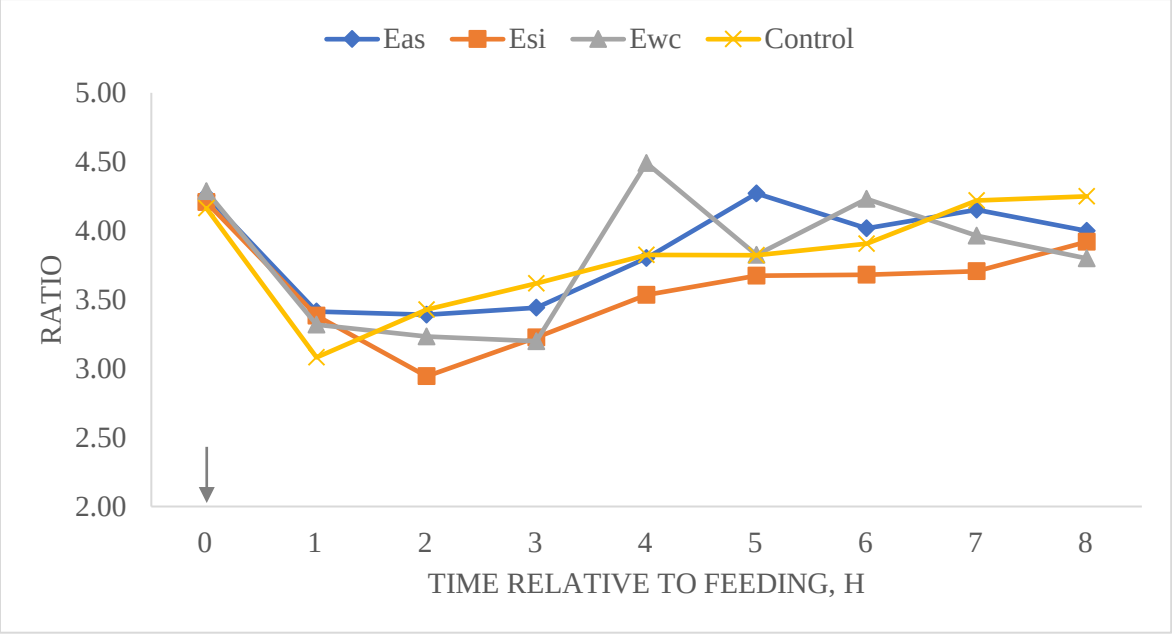

Figure 26. Postprandial ratio of acetate to propionate in dorsal sac of rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period of post feeding. Arrow shows the feeding time at 0700.

4.5.3 Postprandial fermentation in medial region of the rumen

In medial region of the rumen, feeding concentrates Eas and Ewc resulted in higher postprandial pH than feeding the control diet (P<0.05) (Table 14). Total amounts of VFA in medial region of the rumen was not different among the diets (P>0.05). The molar proportion of isobutyrate was decreased by feeding concentrate Esi compared with feeding the control (P<0.05).

Table 14. Postprandial pH, concentrations of VFA and ammonia in medial region of the rumen.

	Experimental concentrates[1]					*P*-Values		
Items	Eas	Esi	Ewc	Control	SEM	Cons[2]	Time	Time×Cons
Medial region:								
pH	6.13[b]	6.17[ab]	6.13[b]	6.24[a]	0.04	0.11	<.01	0.99
Total VFA, mmol/L	102.3	98.0	99.6	97.4	2.43	0.45	<.01	0.64
Acetate, % of VFA	65.4	64.9	65.4	65.2	0.50	0.83	<.01	0.98
Propionate, % of VFA	17.9	18.6	18.1	17.6	0.51	0.29	<.01	0.98
Butyrate, % of VFA	13.4	13.3	13.3	14.0	0.64	0.34	<.01	0.99
Isobutyrate, % of VFA	0.72[b]	0.64[a]	0.67[ab]	0.70[b]	0.04	0.04	<.01	0.93
Valerate, % of VFA	1.39	1.43	1.34	1.39	0.05	0.53	<.01	0.97
Isovalerate, % of VFA	1.19	1.10	1.14	1.09	0.12	0.68	<.01	0.93
Acetate: Propionate	3.70	3.53	3.67	3.76	0.13	0.57	<.01	0.97
Ammonia, mg/L	72.9	72.4	79.7	80.3	13.7	0.90	<.01	0.90

[1] Eas: extruded as such; Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Concentrate.

[3] Means followed by different letters indicate statistical difference as P<0.05.

Patterns of pH, VFA and acetate to propionate ratio in medial region of the rumen

The pH patterns for the concentrates were quite similar, first descending and then ascending (Figure 27).

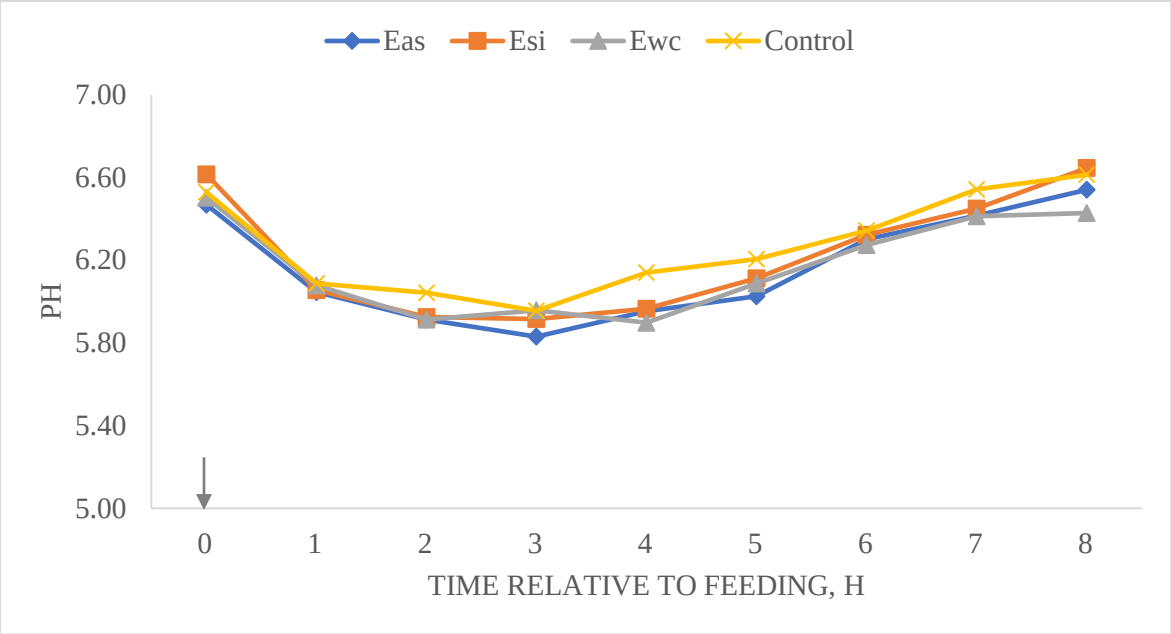

Figure 27. Postprandial pH in medial region of rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

A quick elevation of the amounts of VFA appeared after feeding, followed by a steady decrease, with concentrate Eas increasing to a higher extent compared to the others (Figure 28).

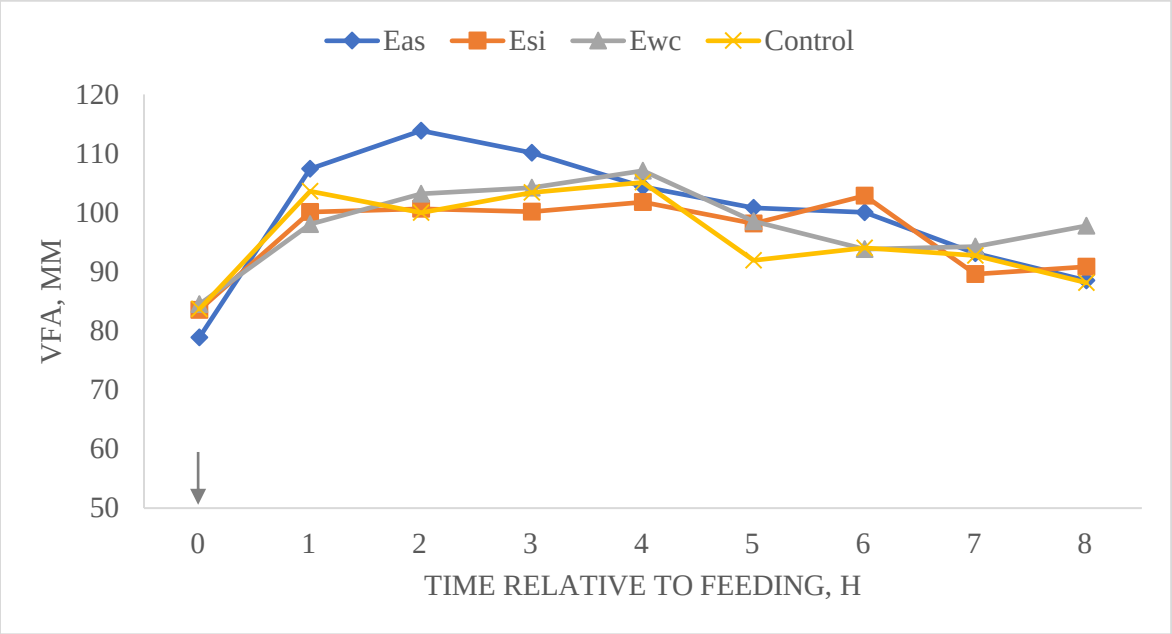

Figure 28. Postprandial total concentration of volatile fatty acids (VFA) in medial region of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

The ratio of acetate to propionate was rapidly going down postprandially with feeding all concentrates, among which feeding concentrate Esi showed a relatively lower nadir than feeding the other concentrates. The ratio started rising 2 h post feeding (Figure 29).

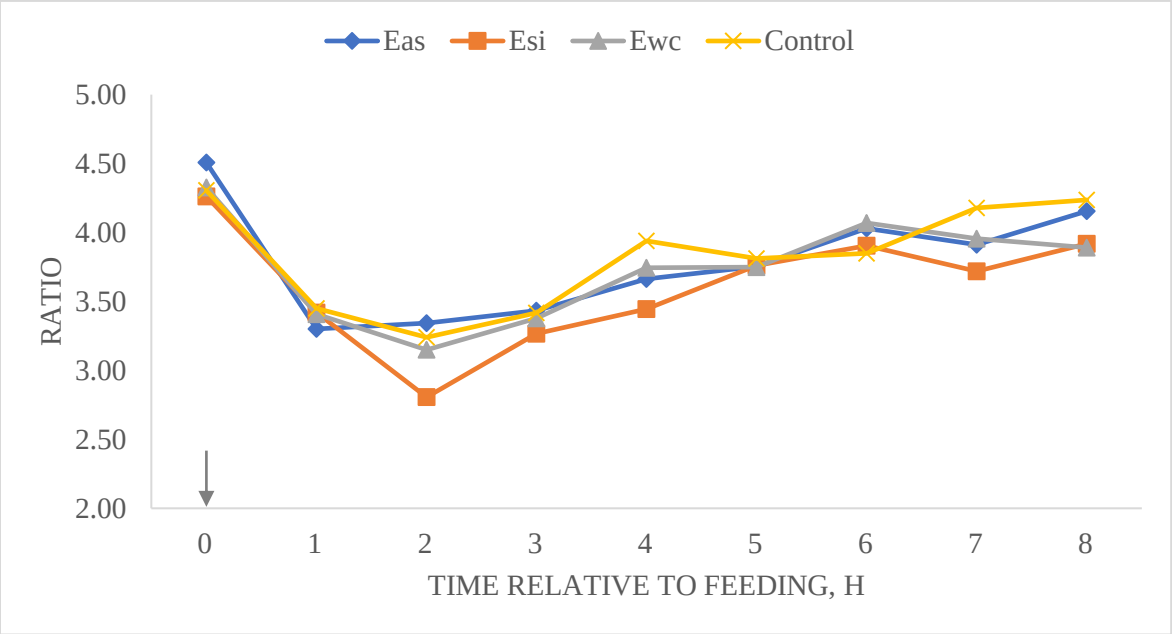

Figure 29. Postprandial ratio of acetate to propionate in the medial region of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period of post feeding. Arrow shows the feeding time at 0700.

4.5.4 Postprandial fermentation in ventral sac of the rumen

Postprandial pH in ventral sac of the rumen did not differ among the concentrates (Table 15). The molar proportion of butyrate was decreased when the cows were fed the extruded concentrates vs the control. Feeding concentrate Esi resulted in the decrease of the molar proportion of isobutyrate compared to the control (P<0.05).

Table 15. Postprandial pH, concentrations of VFA and ammonia in ventral sac of the rumen.

	Experimental concentrates[1]					*P*-Values		
Items	Eas	Esi	Ewc	Control	SEM	Cons[2]	Time	Time×Cons
Ventral sac:								
pH	6.45	6.47	6.45	6.44	0.04	0.92	<.01	0.79
Total VFA, mmol/L	91.7	89.7	89.2	90.6	1.95	0.81	<.01	0.56
Acetate, % of VFA	65.7	65.5	65.8	65.0	0.44	0.61	<.01	0.70
Propionate, % of VFA	17.9	18.6	17.9	17.8	0.52	0.42	<.01	0.94
Butyrate, % of VFA	13.2[b]	13.0[b]	13.2[b]	14.0[a]	0.57	0.09	0.01	0.23
Isobutyrate, % of VFA	0.72[b]	0.64[a]	0.71[b]	0.75[b]	0.04	0.003	<.01	0.85
Valerate, % of VFA	1.32	1.31	1.27	1.35	0.04	0.49	<.01	0.67
Isovalerate, % of VFA	1.18	1.06	1.13	1.22	0.12	0.55	<.01	0.74
Acetate: Propionate	3.73	3.56	3.73	3.70	0.13	0.64	<.01	0.83
Ammonia, mg/L	74.0	76.0	80.1	82.2	11.4	0.87	<.01	0.99

[1] Eas: extruded as such; Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Concentrate.

[3] Means followed by different letters indicate statistical difference as P<0.05.

Patterns of pH, VFA and acetate to propionate ratio in ventral sac of the rumen

All four concentrates resulted in similar pH pattern post feeding, whereas the cows fed the control took more time to reach the nadir than feeding the others (Figure 30).

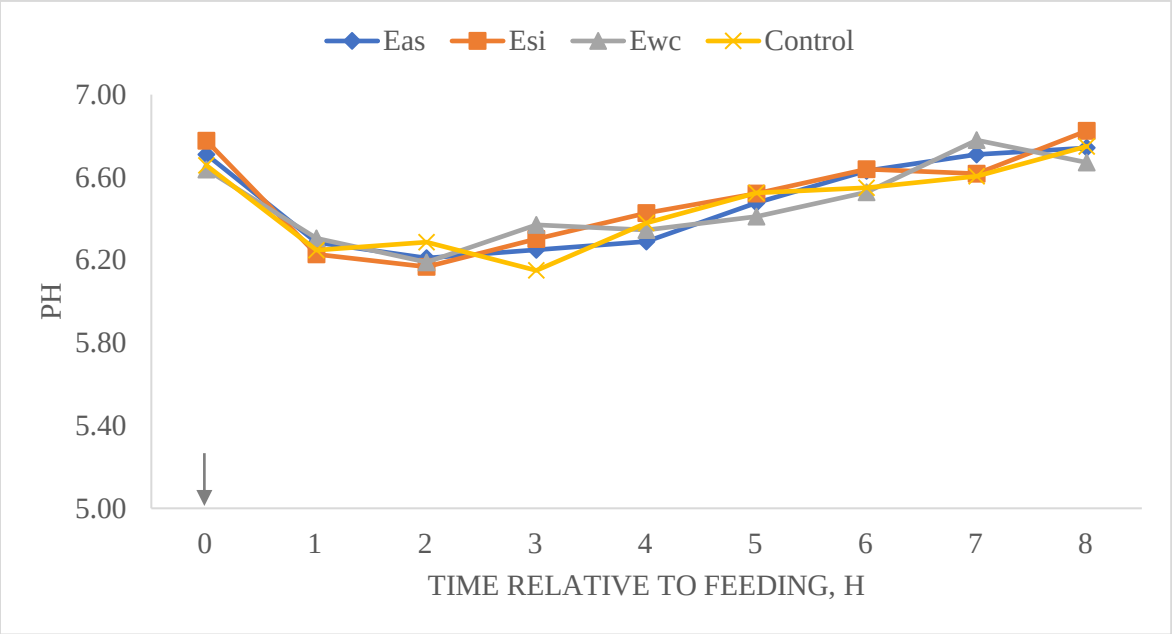

Figure 30. Postprandial pH in ventral sac of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

Feeding concentrate Esi led to a large fluctuation over the 8-h post feeding, showing highest peak 1 h after feeding and lowest nadir 2 h before next feeding (Figure 31). A sudden jump was observed when feeding concentrate Eas 4 h post feeding.

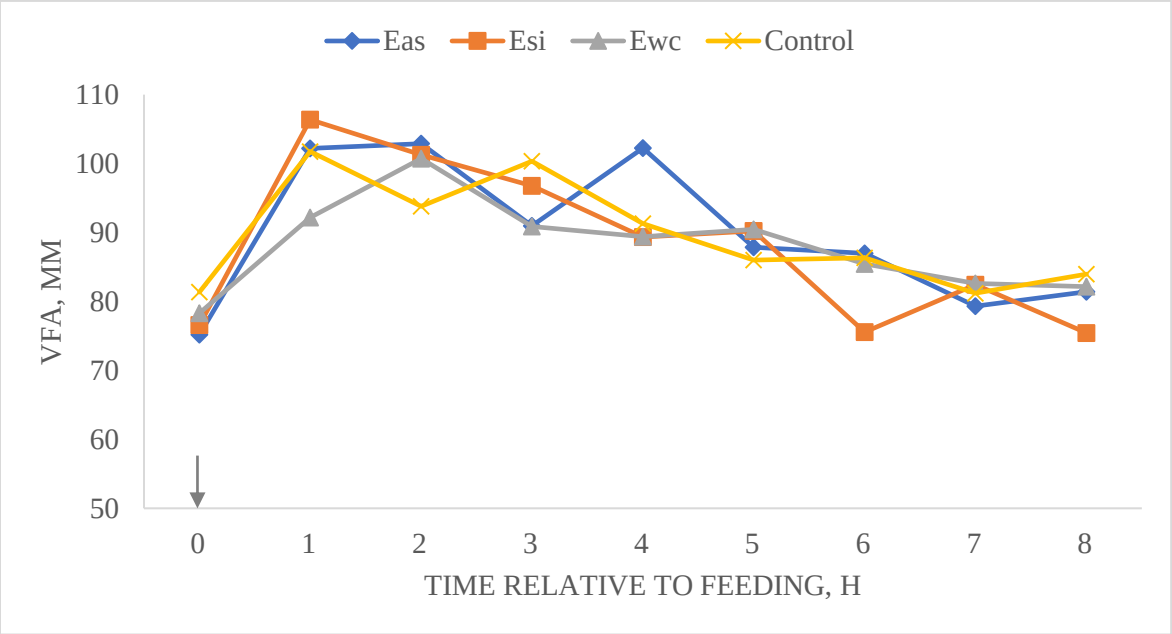

Figure 31. Postprandial total concentration of volatile fatty acids (VFA) in ventral sac of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period. Arrow shows the feeding time at 0700.

For all concentrates, a rapid drop of the ratio of acetate to propionate occurred right after feeding before slowly going up (Figure 32). The ratios for feeding concentrates Ewc and control decreased after reaching the peak when closing to the next feeding.

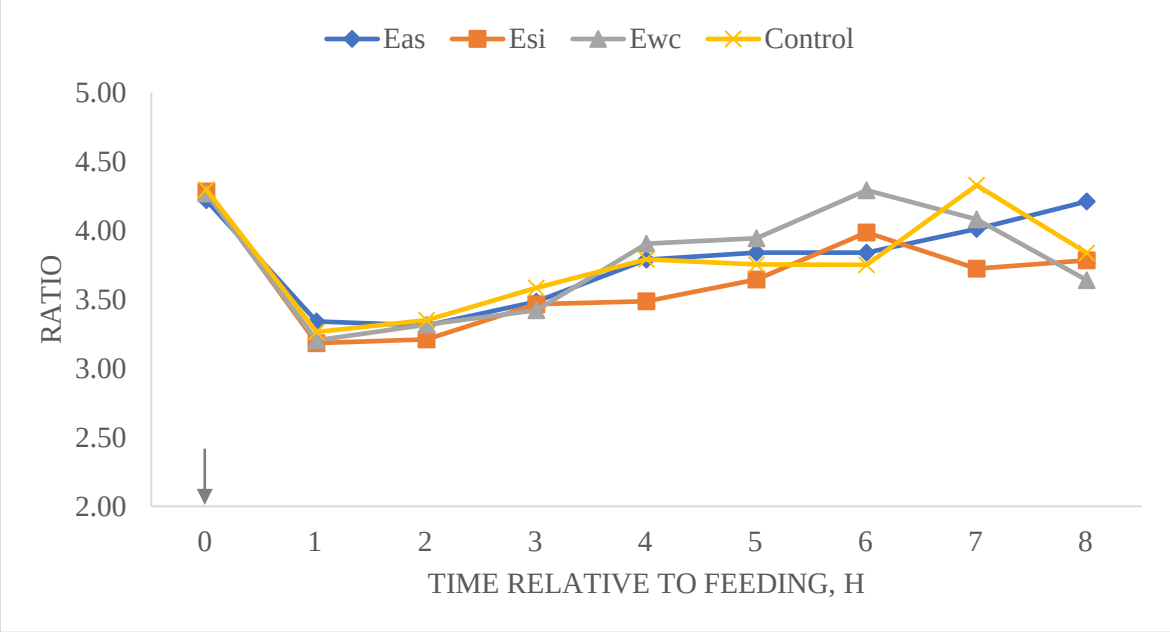

Figure 32. Postprandial ratio of acetate to propionate in ventral sac of the rumen for cows fed the concentrates Eas, Esi, Ewc and control, respectively, measured over an 8-h period of post feeding. Arrow shows the feeding time at 0700.

Overall, the above figures showed that the postprandial patterns of pH and acetate to propionate ratio were similar, decreasing after feeding and then increasing as a function of time, whereas the opposite trend applied to the patterns of the concentration of VFA. The mean postprandial pH in ventral sac of rumen was highest as compared to the other two parts of the rumen. No significant difference of the content of ammonia among the concentrates was observed.

4.6 Feed intake and digestibility

In Table 16, the intake of concentrates was numerically lower for concentrate Ewc compared with the others and the silage intake was highest when the cows were fed concentrate Ewc. The intakes of total dry matter and nutrients did not vary among the concentrates. Rumen digestibility of starch (87.1%) was significantly decreased when the cows were fed concentrate Ewc as compared to the other two extruded concentrates and tended to be lower than that of the control. Rumen digestibility of protein (-42%) and NDF (40.8%) was numerically lowest for concentrate Esi. Total tract digestibility did not differ among the concentrates with respect to DM, protein and NDF. However,

total tract starch digestibility was highest when the cows were fed concentrate Ewc, being greater than when feeding concentrate Esi (P<0.05).

Table 16. Feed intake, rumen digestibility and total tract digestibility (on dry matter basis).

	Experimental concentrates[1]					
Items	Eas	Esi	Ewc	Control	SEM	*P*-Value
Feed intake						
Silage (DM, kg/d)	12.2^{b}	12.4^{ab}	13.4^{a}	12.5^{ab}	0.30	0.13
Concentrate (DM, kg/d)	8.8	8.9	8.2	8.8	0.21	0.17
Total dry matter (kg/d)	21.0	21.3	21.6	21.3	0.41	0.82
Nutrients[2] (g/d)						
Starch	3507	3450	3302	3579	112	0.42
Crude Protein	3230	3355	3262	3374	64	0.48
NDF	7881	8075	8508	8000	215	0.28
Rumen digestibility, %						
Dry matter	24.0	21.4	21.0	24.8	1.88	0.46
Starch	90.6^{b}	91.2^{b}	87.1^{a}	88.5^{ab}	0.70	0.02
Protein	-39.4	-42.0	-40.5	-34.2	3.20	0.41
NDF	47.4	40.8	41.7	47.7	3.50	0.42
Total tract digestibility, %						
Dry matter	67.8	68.6	68.2	68	2.64	0.99
Starch	99.8^{ab}	99.7^{b}	100^{a}	99.8^{ab}	0.06	0.07
Protein	66.0	66.0	66.9	66.9	3.33	0.99
NDF	50.8	52.8	52.3	50.0	3.90	0.93

[1] Eas: extruded as such, Esi: extruded with steam injection into 4th section of extruder, Ewc: extruded with water cooling for the 5th section of the extruder.

[2] Nutrients intake derived from both silage and concentrate.

[3] Means followed by different letters indicate statistical difference as P<0.05.

5. Discussion

5.1 Physical quality

Concentrates from first two groups using the smaller screen size showed large variations on bulk density, whereas concentrates ground on the larger screen size all had similar high bulk densities, irrespective of extrusion conditions. This indicates that the large particles resulting from using the large screen opening (Mani et al., 2006) were too large to be affected, with respect to the bulk density, by different extruding conditions. The reason could be that the large particles absorb less water compared with small particles (Hemmingsen et al., 2008). The less water absorption leads to less flash-off of product moisture when the extrudate leaves the extruder die, thereby leading to the high density of the product (Tumuluru, 2014). Although suggested by Svihus et al. (2004) that more weak points in the pellet due to the large particles could lower the hardness of pellets, group III (concentrates 7-9) did not differ with the other two groups in terms of the hardness. However, the group did have lower WSI than the other two groups. It was plausible that the smaller particles have more surface area than large particles, facilitating moisture and heat penetration (Vegas et al., 2008) and thus undergo a higher degree of starch gelatinization as compared to the large particles. This implied that for large particles there exist less cohesive forces originating from starch gelatinization such as hydrogen bonds between water molecules and hydroxy groups of starch (Ratnayake and Jackson, 2006) to withstand the disruptive forces (Rolfe et al., 2001). Consequently, the lower extent of gelatinization resulted in the lower water stability. The result is in agreement with the results of Svihus et al. (2004) and Rolfe et al. (2001). They claimed that the coarsely ground particles had a very low extent of gelatinization, which generally correlated with a decreased water stability. Moreover, the concentrates which had high densities (concentrates 3, 6-9) resulted in sinking pellets as bulk density determines the extrudate sinking or floating (Sørensen, 2012, Chevanan et al., 2008).

Although group I and group II had similar physical qualities, the experimental concentrates were produced using the same processing parameters as the first group, since the pellet size for the concentrates (4-6) was too large because of the pellets extruded with large die size (5 mm) for practical use according to previous studies. Due to more barley and less SBM used in the processing of the experimental concentrates, concentrates Eas and Ewc both had higher but concentrate Esi had lower bulk density and water stability compared with the counterparts

(concentrates 1-3) in the pilot production (Table 5&6). As expected, concentrate Eas was slow sinking because of the improved bulk density rather than floating as concentrate (1) did (Table 5&6). In accordance with a recent study by Larsen and Raun (2018), these changes were due to the increased starch content in the formula when more barley was added, which may enhance starch gelatinization. Bulk density is negatively affected by expansion ratio. The factor governing the expansion ratio is viscosity (Harmann and Harper, 1973), as it resists the bubble growth driven by the pressure difference between interior of the growing bubble of the extrudate and atmospheric pressure (Ding et al., 2006). When the starch content is improved, the viscosity elevates because of more starch being gelatinized such that the expansion of the pellet was constrained. Therefore, the bulk density increased. Moreover, the elevated water stability may be caused by the increased viscosity, since viscosity is positively associated with the improved binding between particles (Svihus et al., 2005). The decreased bulk density and water stability for concentrate Esi were likely affected by the elevated temperature.

The different conditions applied during extrusion were, in essence, altering temperature exerted on the material in the extruder barrel. From Figure 17 (A), bulk density, hardness and water stability were all inversely proportional temperature. This might be due to the reduction of the material viscosity in the extruder barrel. The excessive heat makes the molecules become vibrated and begin to move. The energy of this movement is enough to untangle the polymers (Aarseth et al., 2006) and overcome the forces binding the molecules together, enhancing product fluidity and thus decreasing its viscosity. As a result, the extrudates become more expanded, resulting in the lower bulk density. The negative relationship between temperature and viscosity has been reported in many studies with respect to extruder treated cereals (Singh and Smith, 1997, Ryu and Ng, 2001, Altan et al., 2008). In addition, lower hardness may be attributed to weak bonds owing to the thin wall of the more expanded pellet. The positive correlation between bulk density and hardness has been demonstrated by Ding et al. (2006) and Altan et al. (2008). A possible explanation for the decreased water stability when increasing temperature is that the pellet with higher degree of expansion due to the high temperature causes more pores in it, allowing more liquid penetrating in and being disrupted more easily. Another reason for the decrease of WSI was proposed by Ding et al. (2006). They postulated that when the moisture content was low (14-22%), leaching or degradation of starch molecules, termed as dextrinization, may prevail over gelatinization with temperature increasing. Hence, more starch will be solubilized due to dextrinization, indicating the

lower WSI. Interestingly, Figure 17 (B, C & D) showed that WSI was proportional to temperature. It is probable that the effect of temperature on viscosity and starch degradation was insignificant when the starch content was low. On the other hand, gelatinization was taking into effect on the improved WSI.

5.2 *In sacco* ruminal degradation

Dry matter degradation

Arieli et al. (1995) reported that in comparison with untreated barley grain, the expanded barley showed reduced EDMD, whereas extrusion had no significant effect. In the present experiment, feeding the extruded concentrates led to lower EDMD as compared to feeding the control (Table 8). This was attributed to significant reduction in the immediately degradable fraction (A) for the extruded concentrates. The decreased A-fraction might be caused by the better WSI thereby reducing the water-soluble components. Figure 19 indicated that ESD and EPD showed inverse relationship with WSI. Starch and protein accounted for the vast majority of DM and most importantly the content of starch was higher than that of protein, which may result in a negative correlation between EDMD and WSI as ESD did. In addition to starch and protein, EDMD could also be affected by degradability of fiber. Singh et al. (2007) reviewed that exposure of insoluble fibrous macromolecules to shear by extrusion led to breakage of chemical bonds thereby producing smaller and soluble particles. Unfortunately, *in sacco* ruminal degradation of fiber was not monitored in this experiment.

Starch degradation

There are some reports on the effects of extruder processing either greatly increasing the rate of disappearance of starch in the rumen (Walhain et al., 1992, Yahaghi et al., 2014) or reducing effective rumen degradability, though the difference was minor (Razzaghi et al., 2016). Few has compared the difference between extruder and expander treatment concerning ESD. One study claimed that the degrees of the reduction of starch degradation were similar for expansion and extrusion processing (Arieli et al., 1995). According to correlation Figure 19, ESD was strongly correlated with WSI ($R^2 = 0.99$), indicating that the high WSI resulted in the low ruminal starch degradability. With respect to the effect of heat input on ESD, the highest temperature resulted in the lowest ESD, which is in line with the results of Larsen and Raun (2018) claiming that extruding at high temperature (115 ^oC) increased starch degradation for most concentrates.

ESD for feeding concentrate Ewc that had the highest WSI was mainly affected by decrease of the immediately degradable fraction (A). This decrease implied that the higher WSI was associated with less loss of the immediately soluble starch owing to high material viscosity evident by high pressure in the extruder die (data not shown). The high viscosity resists the swelling of the extrudate and thus contributes to favorable binding between particles. The general mechanisms for binding particles involve the 'solid-solid' interactions between particles, 'liquid necking' referred to as capillary forces in a three-phase system of water, air and solid material and interactions between particles due to folding and plying (Thomas and Van der Poel, 1996). In addition, the high viscosity favored complex formation in the extruder (Robin, 2001). Complexation between lipids and amyloses, readily taking place during heat processing of starch, appears to influence the rate of enzymatic digestion of starch (Seneviratne and Biliaderis, 1991, Tufvesson et al., 2001). However, the rate of starch digestion in the rumen did not vary among the concentrates, probably because of the negligible effect from the complex. Extrusion can induce chemical reactions between starch granules increasing starch crystallinity which further decrease ruminal starch degradation (Franco et al., 1995). Retrograded starch, formed upon cooling or storage of the gelatinized starch, is difficult to solubilize (Chung et al., 2006). These, however, were insufficient to explain the significant difference of ESD among the concentrates. Maillard reactions within surrounding protein matrix due to heat treatment enable the physical protection of starch from ruminal digestion. But most of studies that found reduced starch degradation in the rumen caused by Maillard reactions would occur at high temperature (Cros et al., 1992, Razzaghi et al., 2016). In the present experiment, the low ESD when feeding concentrate Ewc was obtained at low temperature (79 ^{o}C).

Protein degradation

Contrary to ESD, lowest effective protein degradability (EPD) was obtained at high temperature (125 ^{o}C). According to Figure 18 and 19, EPD was more relevant to temperature (R^2=0.82) than WSI (R^2=0.58). The negative value (Figure 16) obtained for concentrate Esi at incubation of 0 h was difficult to explain, which was probably due to analysis error of 0 h sample. However, the result for significance analysis was unaffected among the concentrates with respect to the degradation characteristics when the negative value was improved to the expected value.

The expander treatment rendering a decrease in protein degradability has been revealed by many studies on various cereals or legumes (Prestløkken, 1999, Goelema et al., 1999, Prestløkken and Harstad, 2001). In this experiment, concentrate Esi with steam injection and thus the resultant high temperature showed even lower EPD than the control (Table 10). The reduction on EPD was mainly ascribed to the decrease of fractional degradation rate (C) under intense heat treatment. This is in agreement with the findings by Razzaghi et al. (2016), who found that extrusion with steam addition reduced EPD considerably in both cereal-protein mixtures (50% maize + 50% SBM, 50% wheat + 50% SBM) due to the reduced rate of protein degradation. It was of interest that according to Figure 18, the correlation coefficient (R^2) did not change between temperature and EPD when the control was involved. It is reasonable to assume that temperature is more important than duration of heat to protect proteins from ruminal degradation (Nowak et al., 2005). The effect of extrusion on determining rumen undegraded protein depends upon both materials and intensity of heat input. Griffiths (2004) reported that extrusion significantly lowered EPD in lupins, full fat soybeans, SBM, canola meal and sunflower meal (20.7% on average), when temperature reached to 120 ^{o}C without causing damage to the protein. Aldrich et al. (1997) observed that when the extrusion intensity was increased (104, 140 and 160 ^{o}C), the degradable fraction of soybeans measured *in situ* decreased (45.7, 36.7 and 30.1% respectively). In the study of Nowak et al. (2005), the highest temperature of extrusion (165 ^{o}C) was effective in protecting soybean protein from microbial degradation in the rumen, though no optimal temperature was determined. However, Walhain et al. (1992) demonstrated that temperature above 140 ^{o}C failed to improve the protection of pea proteins. Thus, an optimum heat input differs from one dietary protein to another.

The reason for the decrease of EPD due to heat treatment might be explained by denaturation and structural rearrangement of protein molecules. Formation of intermolecular disulfides reduced rumen degradation of cystine in peas as well as protein in general (Ljøkjel et al., 2003). Cross-linking reactions such as isopeptide bonds that exist between available amide groups in glutamine and lysine may reduce rumen degradable protein at high heat input (Svihus, 2008). Moreover, lysine, as the primary amino acids in Maillard reactions, played a key role that made feed protein more resistant to rumen degradation (Solanas et al., 2005); these authors concluded that extrusion at 140 ^{o}C significantly reduced N degradation of all protein sources; they also reported that the addition of maize to whole soybeans strengthened the effect on reducing ruminal N degradation. This may be due to the sufficient reducing ends of carbohydrates in maize to induce Maillard

reactions. Hence, the amount of carbohydrates to provide the reducing ends and the intensity of heat input appear to influence the rate and extent of protein degradation in the rumen in the present experiment.

5.3 Rumen fermentation

Feeding extruded concentrates decreased rumen daily pH as compared to feeding the control. It can be speculated that the extruded pellets were floating around in the rumen rather than sinking in bottom for the control as observed in the sinking test. This resulted in more concentrate fermentation taking place above ventral sac and thus caused lower pH as rumen liquor samples were taken from the ventral sac all the way up to the dorsal sac. The amount of time that pH drops below a specific point can indicate fermentation irregularity (Archimède et al., 1997). It has been shown that when pH drops below 6.2 fiber digestibility is reduced (Krajcarski-Hunt et al., 2002). Khafipour et al. (2009) defined SARA as pH below 5.6 for at least 3 h/d. In the present experiment, pH below 6.2 ranged from 63% to 77% of a 24-h period and intake of concentrates and grass silage were not affected. The time spent blow 5.6 were all less than 3 h of a 24-h period, indicating low possibility of suffering from SARA. Overall, as compared to the control, the extruded concentrates spent longer time below all pH points examined, which reflected the lower daily pH for the extruded concentrates. In addition, postprandial pH in the rumen and in dorsal and medial rumen were in general numerically or in some cases statistically lower for the extruded concentrates than for the control, whereas it was opposite in ventral rumen. This was probably due to that the extruded concentrates had better mix with rumen particles as suggested by Nordqvist et al. (2014), while the control was rapidly sinking to bottom of ventral rumen. The variation of individual VFA was complex. The concentration of propionate and butyrate was high and low, respectively, both in rumen and in dorsal rumen for concentrate Esi as compared to the control. This may indicate that concentrate Esi was floating and thus had longer retention time leading to more starch degradation to produce propionate. The low butyrate content for the extruded concentrates in dorsal and ventral sac may be related to less available readily fermentable carbohydrates (Chen et al., 2008). It is notable that concentrate Esi showed decreased concentration of isobutyric acid in all three regions of rumen compared with the other concentrates. This reduction on branch-chained isobutyric acid may be the reason for the decreased ruminal protein degradability (Table 16), which has also been demonstrated by the decrease of EPD. Branch-chained VFA are the product of deamination of branch-chained amino acids, such as leucine, valine and isoleucine (Dehority,

2003). A decreased concentration of branch-chained amino acids has been reported by Mansfield et al. (1994) when low rumen degradable protein were fed.

5.4 Ruminal and total tract digestibility

Ruminal DM digestibility was numerically lower for concentrates Esi and Ewc, but total tract digestibility was greater for them as compared to the other two concentrates, indicating that concentrates Esi and Ewc had higher post-ruminal digestibility. Lowest ruminal starch degradability was observed when the cows were fed concentrate Ewc (Table 16), which was consistent with the results obtained *in sacco* where the magnitude of effective starch degradability was the lowest. The reason for the reduced ruminal starch degradability, as mentioned earlier, could be the best WSI that concentrate Ewc had. Moreover, from the view of the passage rate of point, concentrate Ewc had a higher likelihood of passing out of the rumen because of fast sinking in rumen liquor (high density, 650 g/L) and having high WSI (75% relative to DM). This speculation is derived from a recent study by Larsen et al. (2018). They found conventional pellets with high density (756 g/L) and WSI (81% relative to DM) had the largest ruminal outflow of starch. Additionally, feeding concentrate Ewc resulted in high total tract digestibility. Hence, it is reasonable to believe that the post-ruminal digestibility of starch is the highest for concentrate Ewc. This result is similar to a previous study showing that inclusion of extruded ground corn in the diet increased lower gut digestibility of nonstructural carbohydrates (Shabi et al., 1999). The numerically lowest value of rumen digestibility of protein was observed (-42% on DM basis, Table 16) when the cows were fed concentrate Esi, which indicated that the concentrate had the most protein flowing out of rumen. This is, to some extent, due to the least ruminal protein degradation in the rumen owing to the minimum disintegration rate as observed *in sacco*. However, since the rumen-escaping proteins contain both dietary and microbial protein, the contribution of protein from the concentrate was hard to decide. Thus, further analyses need to be done to determine the amount of dietary protein out of total protein outflow.

6. Conclusion

The effect on bulk density and water stability of concentrates were both dependent on grinding size and conditions applied during extruder processing, whereas the effect of conditions on bulk density was negligible when large grinding size was used. Concentrates with high water stability and different sinking characteristics could be achieved by extruder processing. The extruder treatment reduced rumen dry matter and starch degradation (EDMD and ESD), of which ESD was lowest when the concentrate had highest water stability in line with the lowest ruminal digestibility of starch measured *in vivo*. In agreement with the lowest EPD obtained *in sacco*, concentrate with steam injection showed lowest rumimal protein digestibility. It is difficult to conclude on rumen passage rates based solely on the present study, but it seems that the extruded concentrates had better intra-ruminal mixing with the forage part according to rumen pH measurement. The overall conclusion of the study is that extruder processing can be used to produce feeds that can potentially escape rumen degradation without affecting total tract digestibility, indicating an increase of nutrient digestion in the small intestine. The effect of processing on rumen outflow rate and on nutrient digestibility in the small intestine need further investigations.

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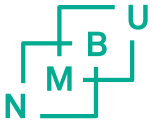

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