The Effect of NaOH-treated Wheat on the Voluntary Intake and Production in Dairy Cows Fed Total Mixed Rations

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Tiivistelmä — Referat — Abstract

Lipeä on vahva emäs, jonka on havaittu lisäävän hemiselluloosan ja ligniinin hydrolyysiä pötsissä. Näin ollen lipeäkäsittelyllä on mahdollista korvata viljan mekaaninen litistys ja jauhatus. Seosrehuruokinnalla, jonka osana on lipeäkäsitelty vilja, on mahdollista vähentää liiallisesta tärkkelyksestä aiheutuvia metabolisia ongelmia pötsissä. Tämän tutkielman tarkoituksena oli selvittää lipeäkäsitellyn vehnän vaikutusta lypsylehmien syöntiin ja tuotokseen *ad libitum* seosrehuruokinnoilla. Ruokinnoissa korvattiin kuivaa murskattua vehnää asteittain kokonaisella lipeäkäsitellyllä vehnällä. Kontrollina oli perinteisesti käytetty kuiva, murskattu ohra-kaura seos.

Koe tehtiin Ruotsin maatalousyliopiston (SLU) maataloustieteiden laitoksella Uumajassa. Koe alkoi syyskuussa ja päättyi joulukuussa 2010. Kokeessa oli 17 useamman kerran poikinutta lehmää ja 6 ensikkoa (Ruotsin punainen -rotu). Lehmät olivat lämpimässä pihattonavetassa, jossa seosrehun syöntiä mitattiin vaakakuppien avulla. Koekäsittelyt olivat murskattu ohra-kaura seos (1:1), murskattu kuiva vehnä (1:0), murskatun kuivan vehnän ja kokonaisen lipeävehnän seos (1:1) ja kokonainen lipeävehnä (1:0). Ruokintojen kuiva-ainepitoisuudeksi asetettiin 370 g/kg ja raakavalkuaispitoisuudeksi 180 g/kg kuiva-ainetta. Näennäinen ravintoaineen sulavuus määritettiin happoon liukenemattoman tuhkan avulla. Typen hyväksikäyttöä arvioitiin laskennallisen typpitaseen avulla. Koe toteutettiin 4x4 latinalaisen neliön koemallin mukaisesti ja käsittelyjen väliset tilastolliset erot testattiin kontrastien avulla.

Kuiva-aineen (P_Q=0,02) ja orgaanisen aineen (P_Q=0,02) syönnit lisääntyivät, samalla kun niiden sulavuudet paranivat korvattaessa puolet kuivasta vehnästä lipeävehnällä. Ruokintojen välillä ei ollut tilastollisesti merkitsevää eroa maitotuotoksessa eikä energiakorjatussa maitotuotoksessa. Maidon rasvatuotos lisääntyi vähän (P_Q=0,04) ja rasvapitoisuus selvästi (P_Q=0,004), kun kuivasta vehnästä korvattiin puolet lipeävehnällä. Kun kaikki kuiva vehnä korvattiin lipeävehnällä, maidon valkuaispitoisuus väheni (P_L<0,001). Samoin kävi maidon ureapitoisuudelle (P_L=0,002).

Lipeäkäsittely ei tuottanut tässä kokeessa taloudellisesti kannattavaa tulosta, sillä maidon valkuaispitoisuus väheni ja syönti lisääntyi maitotuotoksen pysyessä samana. Vehnäruokinnoista paras tuotosvaste saatiin kuivan vehnän ja lipeävehnän seoksella.

Avainsanat — Nyckelord — Keywords
NaOH, vehnä, seosrehu, lypsylehmät

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Tiivistelmä — Referat — Abstract

Sodium hydroxide (NaOH) is a strong base which disrupts the seed coat by partial hydrolysis of hemicellulose and lignin in the rumen. NaOH can substitute for mechanical processing of cereal grains. Using total mixed ration including NaOH –treated grains, is an opportunity to avoid the metabolic problems caused by high dietary starch content. The objective of this study was to determine the effects of various levels of NaOH –treated wheat grains in *ad libitum* total mixed ration diet on feed intake and production of dairy cows. Commonly used oats-barley diet was a control.

This study was conducted at the experimental farm of Swedish University of Agricultural Sciences, Umeå, Sweden from September to November 2010. There were 17 multiparous and 6 primiparous cows in the study (Swedish red breed). The cows were kept in a warm loose house barn and intake of total mixed ration was measured by using scale cups. Experimental treatments were ground barley and ground oats in the ratio of 1:1, ground wheat in the ratio of 1:0, ground wheat and NaOH –treated whole wheat in the ratio of 1:1 and NaOH –treated whole wheat in the ratio of 1:0. All the diets were formulated to have a dry matter content of 370 g/kg and crude protein content of 180 g/kg dry matter. Apparent digestibility of nutrient was determined using acid insoluble ash as a marker. Utilization of nitrogen was evaluated using calculated nitrogen balance. The experiment was conducted according to the 4x4 Latin square designs and the statistical differences between the treatments were detected by contrasts.

Intakes of dry matter (P_Q=0.02) and organic matter (P_Q=0.02) increased in pursuance of their improved digestibility as half of the dried wheat was supplemented for NaOH –treated wheat. There was no significant difference between treatments in milk yield or energy corrected milk yield. Milk fat yield (P_Q=0.04) and concentration of milk fat increased clearly (P_Q=0.004) as half of the dry wheat was substituted for NaOH –treated wheat. By substituting all dry wheat for NaOH -treated wheat, milk protein concentration decreased (P_L<0.001). The same occurred for milk urea concentration (P_L=0.002).

The NaOH –treatment did not result in any economic improvement in this study because concentration of milk protein decreased and intake increased, while milk production remained the same. The best production response was achieved by the mix diet of dried wheat and NaOH –treated wheat.

Avainsanat — Nyckelord — Keywords
NaOH, wheat, TMR, dairy cows

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ABBREVATIONS

AA= amino acids

AAT= amino acids absorbed in the small intestine

AIA= acid insoluble ash

BW= body weight

CF= crude fat

CP= crude protein

CPD= crude protein digestibility

DIM= days in milk

DM= dry matter

DMD= dry matter digestibility

DMI= dry matter intake

ECM= energy corrected milk yield

EFD= efficient fiber degradation

EPD= efficient protein degradation

FE= feed efficiency

iNDF= indigestible neutral detergent fiber

ME= metabolizable energy

MEB= metabolizable energy balance

NDF= neutral detergent fibre

NDFD= digestibility of neutral detergent fibre

OMD= digestibility of organic matter

PBV= protein balance in the rumen

RUP= rumen undegraded protein

TMR= total mixed rations

VFA= volatile fatty acids

VOS= ruminal fluid digestible organic matter

WSC= water soluble carbohydrates

1 INTRODUCTION

Dairy cows have been bred continuously to improve their genetic potential for milk production. However, nutritional requirements of high-productive lactating cows are hardly met using only forage as feed. This is because a large proportion of the energy intake is utilized for milk synthesis. Therefore, the nutrient content of the diet needs to be optimized. By mixing the ingredients of the diet as total mixed rations (TMR) is an opportunity to avoid the metabolic problems caused by high starch content (Reynolds 2006). Approximately 10% of Finnish dairy farms used TMR year 2009 and 18.6% of these farms used commercial concentrates as an ingredient in TMR (Toivonen 2010). Oat/barley mix was used most commonly as a source of cereal grains.

Both metabolic and physical factors of animals regulate digestion and intake of nutrients. The feed intake is mainly limited by the physical fill of the rumen in low productive dairy cows, while metabolic mechanism tends to take over the regulation in high-productive cows. Several factors influence feed intake: they can roughly be divided into animal-, environment- and feed-associated groups. This thesis, however, is mainly focused on metabolic factors through the characteristics of feeds. Therefore, the metabolic regulation has greater impact on feed intake of high productive dairy cows. In general, the content of volatile fatty acids (VFA) in the rumen has a negative influence on dry matter intake (DMI). On the other hand, increased digestibility of neutral detergent fiber (NDFD) increases DMI, based on the high passage rate of the digesta. The proportion of water-soluble carbohydrates (WSC) in a diet improves palatability of feed.

Diets for dairy cows in Sweden and Finland are often based on grass silages in which fermentation has been restricted by acid additives. Digestibility and energy content are often high in early-harvested grass silage and the content of fiber low. Ineffective nitrogen utilization is associated with early cut grasses (Kuoppala et al. 2009). Thus, the digestibility of grass is mostly influenced by growth stage at harvesting. Also, the composition of the diet, stage of feeding and method of conversation are important factors (McDonald et al. 2002). In consequence, feed intake may increase because the feed is faster degraded in the rumen compared with mature grass (Kuoppala et al. 2008). Due to the short retention time in the rumen, feed intake will not be limited by physical fill. In general, the forage intake is dependent on how fast food particles are flowing out from the reticulo-rumen into the small intestine (Van Soest 1994). The flow rate is related to chemical composition of food, particle size and degradation rate of digestible nutrients. The National Research Council in United States (NRC 2001) recommends having at least 25% NDF content in dairy cow diets, on a DM basis.

Wheat grains (*Triticum aesivum L.*), as well as mixtures of barley and oats, are used for dairy cows as energy supplement in the diet. Starch content of wheat grains is usually 600-680 g per kg DM resulting in high concentration of metabolizable energy (ME). Concentrates in the diets have increased total DMI. Starch and WSC are fermented rapidly in the rumen into VFA. This results in fast decrease in rumen pH and affects micro-organisms by decreasing their capacity to ferment fibre (Van Soest 1994). Both wheat grains and silage can influence a rapid production of VFA in the rumen when they are high in starch or sugar, respectively. Too fast starch fermentation into lactic acid can cause metabolic disorders such as acidosis (Owens et al. 1998). Due to decrease in the rumen pH, the digestion of fiber and feed intake will depress (D'Mello 2000). However, by treating grains with alkali, it is possible to shift starch digestion site from the rumen towards the small intestine (McDonald et al. 2002). The postruminal digestion of starch has a greater energetic efficiency than ruminal fermentation, because of a minor loss of energy in methane and heat. According to Ørskov (1986), the loss of energy can vary between 12 and 20% of gross energy.

Processing of grain alters the rate of degradation of nutrients and therefore the nutritional value of feeds can change as well. Cows digest whole cereal grains poorly because of the cutinous nature of the seed husk. Hemicellulose is a major portion of the seed coat of most cereal grains (Berger et al. 1981). Ørskov et al. (1974) showed that treatment of grains with aqueous solution of sodium hydroxide (NaOH) can substitute for mechanical processing. NaOH –treatment increases ash content in feed. This causes a reduction in concentrations of other nutrients. The increase is corresponding to the sodium added. The exact mechanism by which alkali improves digestibility is not clear. Probably, the disruption occurs by a partial hydrolysis of hemicellulose and lignin (Dehghan-Banadaky et al. 2008).

NaOH is a strong base, which is prepared chemically. According to the Finnish food safety authority (Evira 2005), it is allowed to use in most human foods to control pH. In EU, NaOH is allowed to be used in animal feeds to improve digestibility of cereal straw (feed number 6.07; European Commision 1998). The use of NaOH –treatment for other material such as grains may be taken up in EU regulations if the treatment becomes general in the future.

The digestibility improves by disrupting the cell wall when microbial fermentation begins in the rumen (el-Yassin et al. 1991). According to Mcniven et al. (1995) dry matter digestibility (DMD) of barley was similar between NaOH –treated and rolled barley in the total tract. The digestibility of neutral detergent fibre was higher and digestibility of starch was lower in the total tract. Ruminal DMD was reduced about 38% by NaOH –treatment of corn cobs in *in vitro* study (Berger et al. 1979) and about 12.9% (Mcniven et al. 1995) and about 3.6% by NaOH –treatment of barley (Kung et al. 1983) according to *in sacco* rumen incubation studies.

NaOH –treatment has been reported to affect starch digestibility. According to De Campeneere et al. (2006) the digestibility of starch was significantly lower in NaOH wheat diet as compared to rolled wheat (95.2 vs. 98.7%). The reduction was even greater in the barley study of Mcniven et al. (1995) (85.3 vs. 97.3%). They reported also a decrease in digestibility of nitrogen in NaOH –treated barley diet compared to control. Ruminal starch digestibility was reduced approximately 18% by NaOH –treatment. By treating grains with NaOH it is possible to shift starch digestion site from the rumen to the small intestine (McDonald et al. 2002). Van Soest (1994) assesses that about 70-90% of starch is digested in the rumen and about half of the remainder is digested in the small intestine. Thus, the amount of the rumen undegraded starch is normally very low. Swedish Lantmännen recommends that starch intake should be less than 5000 g/day and the starch concentration less than 200 g/kg DM of diet.

According to De Campeneere et al. (2006) NaOH –treatment reduced the degradation rate of the wheat grain according to *in sacco* rumen incubations. NaOH –treated wheat grains have been reported to slow down the passage rate in the rumen compared to control (Ørskov and McDonald 1979). It is also possible that NaOH –treatment could slow down the total feed flow and thus improves the utilization of the diet. O'Mara et al. (1997) obtained that ground corn and NaOH –treated wheat seemed to be similar in terms of ruminal passage rate according to duodenal sampling. They are approximately six times slower degraded in the rumen than rolled grains as determined by *in sacco* method. Consequently, starch with slow degradation rate in the rumen will flow to the lower digestion tract and is degraded in the small intestine. Thus, the source of starch has influence on metabolism of a ruminant.

The NaOH –treatment has resulted in varying effects on DMI. According to De Campeneere et al. (2006) DMI increased in wheat diets and barley diets (Sriskandarajah et al. 1980), while Mcniven et al. (1995) reported no effect on DMI in barley diet and wheat diet (Phipps et al. 2001). According to Reynolds et al. (2001) purified corn starch infused to duodenum up to 2100 g/d did not effect on DMI or faecal starch concentration.

Varying effects of NaOH –treatment on total milk yield has been reported. Sriskandarajah et al. (1980), Mayne and Doherty (1996) and De Campeneere et al. (2006) reported increased milk yield while Mcniven et al. (1995) and Phipps et al. (2001) did not find differences in milk yield or composition. However, some effects have been detected in milk composition. Previous studies show depress in milk fat content when the glucose supply had been increased in small intestine (O'Mara et al. 1997, Reynolds et al. 2001). The reason may be that absorbed glucose has been either oxidized to CO_2 or used for body tissue synthesis (Reynolds et al. 2001). Energy may be used for maintaining body fat and protein retention. When the VFA or long chain fatty acids are not produced, fat synthesis is limited. Ørskov (1986) reported, however, higher fat-corrected milk yield for NaOH -treated barley than for rolled barley. De Campeneere et al. (2006) reported an increase in milk fat yield and protein corrected milk yield. Mayne and Doherty (1996) reported no difference in milk fat concentration between ground wheat and NaOH –treated wheat. The same study resulted in higher molar proportion of acetic acid and lower propionic acid in the rumen for NaOH –treated barley than for rolled barley. This occurs generally with increasing amount of dietary fiber in the diet. Increasing amount of glucose in mammary gland could be used for lactose synthesis. Khalili et al. (2001) reported an increase in milk yield, milk protein and lactose concentrations, when corn was compared with barley supplement in grass silage -based diet.

NaOH –treatment slowed down the decrease of rumen pH after feeding treated wheat rather than ground wheat (O'Mara et al. 1997) and treated barley compared to rolled barley (Mcniven et al. 1995). A too low rumen pH is harmful especially for cellulolytic bacteria. In the same study of Mcniven, ruminal NH_3 concentration was 13% higher on ground wheat diet than on NaOH –treated wheat diet four hours after feeding. A rapid increase in ruminal ammonia content correlated with decrease in ruminal pH. Low concentrations of ruminal NH_3 and blood urea indicate a deficiency of rumen degradable protein. Even so, O'Mara et al. (1997) did not find any difference in blood urea content between ground and NaOH –treated wheat. Because microbial protein synthesis is related to the amount of carbohydrate fermented in the rumen, the deficiency of energy has impact on microbial protein synthesis. When starch degradation site is in the small intestine, microbial protein synthesis is limited due to negative energy balance for microbes.

A capacity of starch digestion and glucose absorption occurring in the small intestine and caecum is not clear. Still, many studies have been conducted often by method of glucose or its precursors (starch or propionate) incubations into the upper duodenum. Infusions of acetic and propionic acids into the rumen have reduced feed intake. On the contrary, butyric acid infusion tends to have no effect on it (McDonald et al. 2002). Propionic acid concentration may increase too extensively and cause limitation of liver metabolism (Ørskov 1986). Still, Sriskandarajah et al. (1980) and Mcniven et al. (1995) did not find any effect of NaOH –treated barley on VFA production or composition in the rumen compared to rolled barley. Insulin hormone controls the carbohydrate metabolism and appetite. It has influence on glucose absorption from the small intestine to the portal vein and further for energy of cells. Increased propionic acid uptake from portal blood by liver stimulates insulin production.

In the rumen, starch is first converted by amylases of rumen microbes to maltose and isomaltose. Then maltases or maltose phosphorylases or 1,6-glucosidases convert them to glucose or glucose-1-phosphate (McDonald et al. 2002). The two enzymes that hydrolyze starch are α- and β –amylase (Van Soest 1994). α-amylase cleaves starch chains randomly. It degrades both amylose and amylopectin. β-amylase cleaves units from the ends of the chains. It degrades amylose but its capacity is limited to the peripheral parts of amylopectin. Amylolytic starch digestion in the duodenum and small intestine is dependent on the amylolytic capacity. Pancreatic juice and gall neutralize slowly feed in the duodenum and that may impair amylase activity when efficiency of hydrolysis will be limited. In normal high fiber diets, most of the carbohydrates are degraded in the rumen and do not reach small intestine. Thus, the excretion of amylase can be lower. The absorption in the small intestine is focused on amino acids (AA). Starch infused into the abomasum limited the capacity of digestion because of the deficiency of pancreatic amylase and short reaction time (Ørskov 1986). In the research conducted with sheep the amount of pure starch was not over 200 g/d. Glucose infused into the abomasum, resulted in absorption of glucose up to 400 g/d. A large amount of fermentative starch in the abomasum is related to a risk of displaced abomasum (Van Soest 1994). According to Reynolds (2006), total postruminal starch digestion averaged 95% of starch flow to the duodenum when postruminal flows were as high as 2 kg/d. This suggests, however, that there is no limitation for starch digestion.

Starch can be fermented in the large intestine to VFA as in the rumen, but microbial protein produced would be lost as endogenous excretion (Reynolds 2006). Amino acids cannot be absorbed through the lumen of the large intestine and subsequently apparent nitrogen digestibility is decreased, shifting nitrogen excretion from the urine to the faeces. A nitrogen loss in faeces increases also when starch is fermented in the caecum, where microbial protein is synthesized from residual digesta nitrogen and urea nitrogen. Consequently, urinary nitrogen excretion reduces. Apparent digestibility of dietary nitrogen in this occasion will decrease but has no effect on amount of absorbed amino nitrogen. According to Ørskov et al. (1970) starch can be fermented in caecum and colon up to 100 g/d.

Previous studies with forage indicate that NaOH-treatment is an efficient method to protect not only starch but also protein (Mcniven et al. 1995, De Campeneere et al. 2006). Thus, the rumen undegraded protein (RUP) increases. Although NaOH – treatment decreased the ruminal degradation of crude protein (CP) in barley (Dehghan-Banadaky et al. 2008) and wheat (De Campeneere et al. 2006), the total digestibility of CP was equal to control (De Campeneere et al. 2006). Dehghan-Banadaky et al. (2008)

reported increased post-abosamal tract digestibility of some amino acids (AA) by NaOH –treatment of barley. Disappearance of methionine and glycine increased in total tract using a mobile nylon bag technique. The content of lysine (Mcniven et al. 1995) and cysteine decreased in barley grains using a nylon bag technique (Dehghan-Banadaky et al. 2008). The reduction is explained by the formation of cross-linked AA, lysinoalanine familiar to soybean meal treated with NaOH. In addition, total vitamin E content has been reported to decrease in NaOH -treated barley (Mcniven et al. 1995).

The postruminal starch digestion has affected energy balance at the expense of ruminal microbial protein synthesis. Microbial protein, however, is usually the most important source of essential amino acids and α-amino nitrogen supply for ruminants (D'Mello 2000). According to Reynolds et al. (2001) duodenally infused corn starch did not influence on milk protein content, but decreased urine nitrogen output and increased nitrogen output in faeces. Milk urea nitrogen reflects feed nitrogen utilization. Nousiainen et al. (2004) found an interaction between dietary CP and milk urea nitrogen. Milk urea nitrogen has decreased in corn supplemented diet compared with barley on grass silage based diet (Khalili et al. 2001).

Relatively few studies have evaluated the effects of alkali-treated cereal grains in the TMR feeding system. The diets that have contained NaOH -treated roughages fed to lactating dairy cows have resulted in varying effects on production. Planning of the current study was based on the Danish research of De Campeneere et al. (2006). They found the highest bypass effect of cereals with NaOH –treated wheat. They reported higher DMI and milk performance for NaOH –treated wheat than for rolled wheat. The present study aimed to explore an alternative for an optimized diet for dairy cows in Northern Europe. The objective of this study was to determine the effects of various levels of NaOH –treated wheat grains in a total mixed ration diet on feed intake, nutrient digestion and production of dairy cows during late lactation.

2 MATERIALS AND METHODS

2.1. The place and time of the study conducted

This study was conducted at an experimental farm of Swedish University of Agricultural Sciences, Umeå, Sweden (63°45´N, 20°17´E) from September to November 2010. The loose house barn had two separate sections, a warm and cold section. The cows used in this study were placed in the warm section. All cows were loose but their feed consumption was controlled by specific feeding cups (RIC-system, Insentec B.V. Marknesse, The Netherlands) so that each group of animals had access only to a particular section with the aid of the transponders. This ensured the right diet for the right animals. The feed was distributed as TMR *ad libitum* and the cows had free access to water. The cups were equipped with weights and the system registered the time of eating and amount of feed intake.

2.2. Preparation of feeds

The wheat grains (*Triticum aesivum L.*) were treated with sodium hydroxide to shift degradation towards the duodenum. By this method, it is possible to increase feed consumption and feed utilization by reducing the loss of energy as methane or heat in the rumen digestion. The NaOH –treatment of wheat was prepared by a modified method of De Campeneere et al. (2006). Wheat treated with NaOH was prepared by adding fine-grade NaOH (30g/kg) to whole dried wheat grains (140 g of DM/kg). This preparation was mixed for 2-3 minutes and after this 400 liters of water was added. Mixing continued for 10-15 minutes until the solution was absorbed into the grains. The mix was spread to a 20-40 cm high carpet and cooled down for 24 hours. NaOH-treated dry wheat was stored for 6-9 months in a silo and spread to a 2 m high carpet. The treated wheat was mixed again with water when feeding. 3800 kg of 3% NaOH-treated dry wheat was mixed with 600 l of water for 2-3 minutes.

Grass dominated by timothy (*Phleum Pratense* L.*)* was early-harvested grass and all dietary treatment diets were based on it. The seed mixture contained 20% of red clover, Betty, and two varieties of timothy, Jonatan 40% and Grindstad 40%. Grass was harvested from three fields on 17.6.2010. Both Field 1 (6.65 ha) and Field 2 (9.03 ha) were sowed in 2009 and Field 3 (16.3 ha) in 2007. Grass was sowed with barley on every field. Grass was cut by a mower (Kverneland, working breadth 3.6 m) and picked up and chopped with a precision chaff-cutter harvester (JF ES 5000 Meta Q Protec, volume 50 m^3). The preservative used was Promyr® XR 630 and the amount of preservative used was 3.5 l/ 1000 kg of fresh matter. Promyr® XR 630 is a mixture of formic acid and propionic acid. Propionic acid is often used for inhibiting the growth of mold in cereal grains and total mixed rations. Grass herbage was compacted in bunker silo by a tractor (weight 14 000 kg) and crossed four times per 30 cm of grass material. The silo was covered with plastic and woodchips. The silo was 5.9 x 3 x 23 m of size.

2.3. Animals and management

There were 23 dairy cows of Swedish red breed in the study, placed in seven replications of a 4 x 4 Latin square design. Seventeen of the cows were multiparous, averaged 147 days in milk (DIM) (SD 48) and their daily milk production averaged 28.4 kg (SD 5.01) in the beginning of the study. The 6 primiparous cows averaged 159 DIM (SD 41) and their daily milk production averaged 26.0 kg (SD 1.41). The order of treatments in the design was randomized. The cows were allocated to treatments depending on their parity, DIM and milk production. The cows were kept in warm loose house barn throughout the experiment.

Treatments consisted of four complete diets that had the same percent of forage to concentrate of 52:48 on DM basis. However, they differed in the main energy source. The four treatments contained barley and oats in the ratio of 1:1 (O/B), dried wheat in the ratio of 1:0 (DW), wheat and NaOH –treated wheat in the ratio of 1:1 (SW/DW) and NaOH –treated wheat in the ratio of 1:0 (SW). All the diets were formulated to have a DM content of 37% and CP content of 18% in a dry matter basis. The mix of oats and barley as well as wheat were ground and stored in tower silos. All the diets were supplemented with rapeseed meal to complete nitrogen and mineral requirements. The rapeseed meal (Brassica napus L. 49 and Brassica campestris L.) was a commercial pelleted product of a 00-variety that is low in glucosinolates and tannins.

The feeding interval affects the rate of feed degradation. The cows were fed twice daily at 0800 and 1700 into the feeding cups, which were allocated in a row and six cows shared four cups. Body weight (BW) was measured individually daily after milking and recorded automatically. It represents protein and fat deposition in the body tissue. Average BW of multiparous cows was 655 kg (SD 49) and that of primiparous cows 548 kg (SD 39) at the beginning of the study. The cups were emptied daily and cleaned once per week. Refusals were not allowed to be less than 5 kg per day. Feeds were offered as TMR *ad libitum* and the cows were provided constant access to water. The TMR mixer (Nolan A/S, Viborg, Denmark) was mixing the components with rotary paddles for seven minutes. Each animal continued to the next diet within periods (Table 1).

Table 1. Design of the study.

				Lactating dairy cows[a]			
Period	Start	Stop	Length d	Alfa	Beta	Gamma	Delta
I	2.9.2010	22.9.2010	21	B(DW)	A(O/B)	D(SW)	C(SW/DW)
II	23.9.2010	13.10.2010	21	C(SW/DW)	B(DW)	A(O/B)	D(SW)
III	14.10.2010	10.11.2010	28	A(O/B)	D(SW)	C(SW/DW)	B(DW)
IV	11.11.2010	1.12.2010	21	D(SW)	C(SW/DW)	B(DW)	A(O/B)

[a]Each cow in a square attends all the treatments according to the 4x4 Latin square design, O/B=oats/barley, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat.

2.4. Collection and analysis of samples

2.4.1. Feed samples

The nutrients were analyzed according to NorFor (2010). Both silage and concentrates were analyzed for DM, CP, crude fat (CF), NDF, indigestible NDF (iNDF), starch, WSC, ash and acid insoluble ash (AIA). In addition, silage samples were analyzed for pH, NH_4-N, lactic acid, VFA and ethanol.

The feed samples were collected representatively during the sample collection week, which was the last week of each period. Each period was planned to last three weeks. Due to a technical problem with the feeding equipment, the third period lasted a week longer (Table 1). Feed samples were collected from individual feed ingredients and from TMR as mix samples. The determinations of DM and ash were conducted in a laboratory of Umeå University, SLU. DM1 determination was conducted by drying fresh silage samples in 60°C for 48 h (NorFor 2010). Thereafter, the samples were pooled and milled through a 2 mm screen in a cutting mill (SM 2000, Retsch GmbH, HAAN, Germany). A part of this fraction was milled through 1 mm screen for DM2 and ash determinations. DM2 was determined by drying 2.0 g of sample in a crucible in 105°C for 16 h in duplicate. Taking a representative sample on TMR is problematic because the ingredients assort and the sample, therefore, is not uniform quality. Ash determination was made in 550 °C for 3 h.

Total DM content was calculated according to instructions given by NorFor (2010).

$$(1)\ \mathrm{DM} = \mathrm{DM1} \times \mathrm{DM2}$$

The reconstituted samples were carefully mixed (hand agitation) and analyzed for concentration of CP (AOAC. 1984; method 7.015), neutral detergent fibre (NDF) with addition of sulphite and amylase (Chai and Udén 1998), WSC (Larsson and Bengtsson 1983) and starch (Larsson and Bengtsson 1983). Indigestible NDF concentrations were determined in triplicate by incubating samples in the rumen for 288 h using nylon bags (12 µm pore size and effective surface areas of 100-200 cm^2) and then determining the NDF concentration of the residues using the same procedure as for the other NDF concentrations. Solubility of NDF in the rumen is described as EFD –value (efficient fiber degradation).

2.4.2. Urine and faecal samples

The faecal samples were collected during the sample collection week in three days. Two separate faecal samples, pooled and single were collected straight from rectum. The animals were separated to a stall after milking for sample taking. The single samples were collected from each and every cow in the trial. Both urine and pooled faecal samples were collected from cows in the second (or third) square. By this method, it is possible to evaluate the differences within the cow between consecutive days. The single and pooled faecal samples (250 ml fresh matter) were placed into aluminum container and dried in 60ºC for 48 h. The next sample was spread to the top of former pooled sample and dried the same way, so that there were samples from two days. The samples were milled over a 1 mm screen in a cutting mill (SM 2000, Retsch GmbH, HAAN, Germany). They were analyzed for content of N, NDF and iNDF using near infrared spectroscopy (NIRS) at Valio research laboratory (Valio, AS, Finland). The urine samples were collected by using spontaneous urinates stimulated by tickling the caudal udder with straws. Approximately 250 ml urine was gathered in a test tube only from the same cows as the pooled faecal samples were gathered. Urine was directly frozen in -20°C for later analyses. It was analyzed only for nitrogen in order to measure total volume because there are some references of increased volume associated with NaOH-treatment.

2.4.3. Feed intake and milk production

A ration of concentrate to silage and milk yield are factors that partly explain variation in dry matter intake (DMI). On the other hand, intensive starch degradation in the rumen can influence not only appetite but also cause severe metabolic disorders by lowering rumen pH. The distribution of feed was organized by using individual feed cups. The Roughage Intake Control™ feeder (Insentec B.V. Marknesse, The Netherlands) registered the ratio of concentrate to silage, DMI and time intervals of intake of each animal. The diets were delivered according to pretrial observations of DMI and adjusted to *ad libitum* level feeding. They were mixed once a day in the complete diet feeder. The feed was analyzed in the end of every period to ensure the composition of feed at preceding period.

Individual milk yield was automatically recorded at every milking using a gravimetric method (S.A. Christensen & CO, Kolding, Denmark). The composition was determined in the end of each period on two consecutive days using a pooled sample of the two recordings, morning and evening recordings separately. The cows were milked at approximately 0600 and 1500 h. The samples were stored in 4°C and sent to a local dairy (Norrmejerier).

2.5. Calculations of the results and statistical analysis

Feed values were calculated according to Swedish feed table (2003). In addition, the feed table was used for completing the contents of minerals. The feed evaluation system for bovine diets is based on metabolizable energy (ME) both in Finland and Sweden (Formula 2). The content of ME in feeds was calculated using VOS value (ruminal fluid digestible organic matter). The VOS value was determined by in vitro method of Lindgren (1979). The VOS value was also used in calculations of the organic matter digestibility (OMD, Formula 4).

$$(2)\ \mathrm{ME} = 0.16 \times \mathrm{VOS} - 1.91$$

Protein evaluation was based on AAT- PBV system. The system is based on the results of protein metabolism studies. Protein synthesized in the rumen by microbes and the RUP, are the most important sources of protein in ruminants. The EPD value (efficient protein degradation) was used for calculating AAT and PBV values. The EPD value is defined as a proportion of the CP degraded in the rumen. The EPD value is defined after animal studies for a particular feed. It is determined using a rumen outflow rate of 8% per hour and sample taking after 2, 4, 8, 16, 24 and 48 hours of nylon bag incubations. AAT and PBV calculations are based on publication of Lindgren (1979). The AAT means the amino acids absorbed in the small intestine. This fraction is utilized for production and maintenance. The PBV is the protein balance in the rumen and indicates the sufficiency of the rumen degraded protein and the ratio of protein and energy. Nitrogen was measured using the Kjeldahl procedures.

Apparent diet digestibility was determined using acid insoluble ash (AIA) as an internal marker (Van Keulen and Young 1977). Digestibility of DM was calculated according to formula 3 below. Digestibility of NDF, CP and starch were calculated according to formula 4.

$$(3)\ \mathrm{DMD} = 1 - \frac{\text{concentration of AIA in diet}}{\text{concentration of AIA in faecal DM}}$$

$$(4)\ \mathrm{OMD} = 1 - \frac{\text{concentration of AIA in diet}}{\text{concentration of AIA in faeces}} \times \frac{\text{concentration of OM in faeces}}{\text{concentration of OM in diet}}$$

It has been suggested that NaOH –treatment increases urine volume (Bannink et al. 1999). The amount of water intake was not measured. Formula for calculating urine output volume is presented in Formula 5. The formula takes into account the body nitrogen retention.

$$(5)\ \text{Urine output volume} = (\text{N intake} - \text{milk N} - \text{faecal N} - \text{Nbal}) / \text{urine N concentration},$$

where Nbal=nitrogen balance. In calculating N balance, nitrogen retention was calculated using metabolizable energy balance (MEB). The ME requirement was calculated according to Swedish feed table (2003). The equation lies on the assumption of energy retention in body tissues and protein content in it.

$$(6)\ \text{If MEB} > 0 \text{ then NBal} = \text{MEB}/34 \text{ x } 160 \text{ x } 0.16$$

$$(7)\ \text{If MEB} < 0 \text{ then NBal} = \text{MEB}/(-28) \text{ x } 160 \text{ x } 0.16$$

Milk samples were analyzed for fat, protein, lactose and urea at Eurofins Steins Laboratorium SB (Jönköping, Sweden, Eurofins 2010), using a CombiFoss 5000 MilkoScan infrared technique. Energy corrected milk is calculated according to Sjaunja et al. (1990) as follows:

$$(8) \text{ECM (kg/d)} = \text{milk yield (kg/d)} \times \left(\frac{383 \times \text{milk fat (\%)} + 242 \times \text{milk protein (\%)} + 783.2}{3140}\right)$$

2.5.1. Statistical analysis

There were 23 cows in the study, making up 92 measurements in total. From these measurements 10 measurements were invalidated, because they did not fit on the normal range of feed efficiency (FE) value (Britt et al. 2003). The normal range is 1.3-1.7 kg energy corrected milk yield (ECM)/ kg DMI. Biologically impossible results were formed from four cows (the invalidated results by cow number and period were as follows: 1364 I,II,III,IV; 1345 II,III,IV; 1297 I; 438 I,II).

Data was analyzed by ANOVA for Latin square design according to GLM procedure of SAS, using the following model:

$Y_{ijkl}=\mu+S_i+P_j+C_k(S_i)+T_l+\varepsilon_{ijkl}$, where Y_{ijkl} is the independent variable, μ the overall mean, S_i the effect of square (i=1 - 7), P_j the effect of period (j=1 - 4), $C_k(S_i)$ the effect of a cow within the square I, T_l effect of treatment (l=1 - 4) and ε_{ijkl} is the residual error. The experimental unit is a cow in a period and the design is called change over design, as described by Morris (1999). The interactions period*feed, square*feed and square*period were tested statistically to find out if we can leave these interactions out of the statistical model. The result was that there was no major significant interaction between these variables. Results are presented as least square means (LSM) with standard error of the means (SEM). Post-ANOVA differences between the treatment means were detected by contrasts testing the difference between the grains (O/B vs. DW) and the probability of linear and quadratic responses to increasing amount of NaOH –treated wheat in the diet. All statements of significance are based on the probability level of 0.05.

3 RESULTS

3.1. Feed ingredients and diet composition

Good quality grass silage is the base of a ruminant diet. DM content in feed affects silage quality. The DM content in wilted grass is normally 250-350 g/kg. The ensiling succeeds when conditions are anaerobic, which enable lactic acid fermentation. Lactic acid, produced by microbes, decreases the pH. A proper pH is 3.7-4.0 to limit the growth of detrimental micro-organisms. Good quality grass silage contains 50-150 g of WSC, 20 g VFA and 35-60 g lactic acid, per kg DM. On the other hand, a too low pH decreases intake of the feed. In this study, pH was slightly too high (Table 2). A too low sugar content combined with a high VFA content indicates problems in fermentation during preservation. A too high concentration of lactic, formic, acetic and butyric acid decreases feed palatability and protein value of feed. Enzymes in grass start to degrade protein after harvesting and the end-product from this reaction is ammonium nitrogen. The ratio of ammonium nitrogen to total nitrogen should be less than 80 g/kg N. The NH_4-N concentration was too high and indicates extensive fermentation. Also the amount of soluble nitrogen per total nitrogen indicates protein degradation in silage. This is less than 400 g/kg N for a good quality silage. A proper concentration of CP is 140-170 g/ kg DM. The nitrogen fertilizers, time of mowing and plant species have effect on it. The amounts of nitrogen fertilizer were 80 kg for the first harvest and 40 kg for the second. A high concentration of butyric acid is an indicator of a fermentative error. In this study, butyric acid concentration was more than five times higher than recommended. This has a remarkable effect on silage quality. A good quality of silage contains less than 1g butyric acid per kg DM (Swedish feed table 2003).

The chemical composition of the feed ingredients and diets is presented in Table 2 and Table 3, respectively. The oat and barley contain naturally less starch than wheat. Oat has especially high content of DM compared to barley or wheat. Due to the seed coat oat has high fiber content. The content of CF is higher in oat that in other grains. Both oat and barley contain less starch than wheat. Even so, the starch concentration of wheat was not especially high. Oats/barley diet contained 5 g more of CF, 35 g more of NDF and 15 g more of iNDF, respectively, than DW on DM basis. The diet contained 45 g/kg DM less of starch than DW diet. The NaOH –treatment increased the content of NDF and ash in wheat grains. The treatment decreased the content of iNDF, starch and WSC in the feed material. The treatment did not affect CP, CF or ME concentrations.

Table 2. Chemical composition of the feed ingredients (g/kg DM).

Item	Grass silage	SD[a]	O/B[b]	SD	DW[c]	SD	SW[d]	SD	RSM[e]	SD
Dry matter (g/kg)	231	3.6	869	3.5	878	3.2	662	22.3	892	5.6
Crude protein	171	2.8	130	1.6	141	1.4	141	2.4	345	5.8
Crude fat	26.4	.	40.0	.	22.4	.	19.8	.	80.6	.
NDF[f]	500	10.9	246	.	129	.	164	27.2	318	.
iNDF[g]	88.5	.	76.6	.	24.0	.	5.8	.	234	.
iNDF (g/kg NDF)	177	5.5	312	.	186	.	35.4	.	735	.
Starch	16.5	11.70	488	13.7	637	18.2	601	10.9	20.0	6.20
WSC[h]	1.3	0.40	16.3	.	26.7	.	10.5	.	86.2	.
Ash	84.4	2.10	28.9	1.60	21.4	0.60	67.1	1.40	89.8	2.90
AIA[i]	19.8	0.80	7.9	1.60	0.4	0.10	2.3	0.60	1.3	0.20
Feeding values										
ME[j] (MJ/kg DM)	11.0	0.20	12.4	0.00	14.0	0.00	13.3	0.00	12.5	0.10
AAT[k]	71.4	0.50	79.3	0.20	95.0	0.00	90.7	0.10	201	2.5
PBV[l]	47.2	2.70	-2.7	1.35	-12.6	1.50	-5.4	2.60	62.4	2.50
Fermentation quality of silages										
OMD[m] (g/kg)	765	11.4								
pH	4.2	0.10								
NH_4-N (g/kg N)	86.8	9.76								
Lactic acid	67.3	5.20								
Aceticacid	33.8	2.60								
Propionic acid	2.2	0.20								
Butyric acid	5.8	1.60								
Ethanol	24.8	4.70								

[a]Standard deviation of the mean, [b]oats/barley, [c]Dried wheat, [d]NaOH-treated wheat, [e]Rapeseed meal, [f]Neutral detergent fibre, [g]Indigestible neutral detergent fibre determined using nylon bag technique, [h]Water soluble carbohydrates, [i]Acid insoluble ash, [j]Metabolizable energy, [k]Amino acids absorbed in the small intestine, [l]Protein balance in the rumen, [m]Predicted organic matter digestibility according to Lindgren (1979).

Table 3. Chemical composition of TMR diets (g/kg DM).

Item	Diets[a] O/B	SD	DW	SD	SW/DW	SD	SW	SD
Contribution								
Grass silage	520		520		520		520	
NaOH treated wheat	0.0		0.0		170		340	
Ground Wheat	0.0		340		170		0.0	
Ground Barley/Oat	340		0.0		0.0		0.0	
Rapeseed meal	140		140		140		140	
Composition								
Crude protein	182	1.5	185	2.1	185	2.3	185	2.3
Crude fat	38.5	0.20	33.1	0.75	32.2	0.10	31.9	0.15
NDF[b]	389	5.9	354	8.3	353	6.7	361	9.4
iNDF[c]	74.1	1.47	57.7	2.86	53.4	1.47	51.4	1.58
Starch	175	9.6	220	7.9	222	5.6	213	5.2
WSC[d]	18.3	0.24	21.4	0.55	19.2	0.36	16.8	0.70
Ash	66.8	0.66	64.5	1.03	70.7	1.96	77.9	3.01
AIA[e]	13.1	0.56	11.0	0.64	10.9	0.36	11.3	0.34
Feeding values								
ME[f] (MJ/kg DM)	11.7	0.09	12.2	0.15	12.1	0.08	12.0	0.06
AAT[g]	92.3	0.21	97.2	0.59	96.9	0.26	96.2	0.34
PBV[h]	32.6	1.37	29.6	2.27	30.4	2.09	31.5	2.07

[a]O/B=oats/barley, SD=standard deviation of the mean, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, [b]Neutral detergent fibre, [c]Indigestible neutral detergent fibre determined using nylon bag technique, [d]Water soluble carbohydrates, [e]Acid insoluble ash, [f]Metabolizable energy, [g]Amino acids absorbed in the small intestine, [h]Protein balance in the rumen.

The compositions of diets are presented in Table 3. Wheat diets contained almost the same amount of CP, NDF, ME, AAT and PBV. In general, the wheat diets contained on average 185 g of CP, 356 g of NDF and 12.1 MJ of ME in diet DM. Crude fat, iNDF and WSC concentrations decreased in wheat diets by increasing the NaOH –treated wheat in the diets. Ash content increased with increasing proportion of NaOH –treated wheat in the diets. Concentration of starch was highest as half of the wheat was substituted for NaOH –treated wheat and lowest as the dry wheat completely substituted for NaOH –treated wheat.

3.2. Nutrient intake and digestibility

Intakes of CF, NDF, iNDF, AIA and PBV were significantly higher in O/B diet than in DW diet. On the contrary, intakes of WSC, starch and AAT were significantly lower in O/B than DW diet. There were no significant differences in intakes of DM, OM, CP or ME between the diets. Digestibility of DM, OM and starch were significantly lower in O/B than DW. Digestibility of NDF and CP did not differ significantly between the O/B and DW diets. The O/B and DW were similar in faecal composition except for CP concentration, which was significantly higher in DW.

DMI (P_Q=0.02) and OMI (P_Q=0.02) increased when half of the dry wheat was substituted for NaOH –treated wheat (Table 4). In addition, intake of CP, starch, ME and AAT increased in a quadratic manner. Intake of PBV increased linearly, with increasing amount of NaOH –treated wheat in the diet. Intake of iNDF decreased linearly. Intake of WSC, on the contrary, increased, as half of the dry wheat was substituted for NaOH –treated wheat but decreased as all of the dry wheat was substituted for NaOH –treated wheat.

Table 4. Least square means for intake and apparent digestibility of nutrients by lactating cows.

	Diets[a]					Significance of the contrasts[b]		
Item	O/B	DW	SW/DW	SW	SEM	O/B vs. DW	L	Q
Number of observations	20	20	21	21				
Intake, kg/d								
Dry matter	21.5	21.3	22.2	21.4	0.30	NS	NS	*
Organic matter	20.0	19.9	20.6	19.8	0.28	NS	NS	*
Forage (kg DM/day)	11.3	11.2	11.6	11.3	0.16	NS	NS	NS
Concentrate (kg DM/day)	10.2	10.1	10.7	10.2	0.15	NS	NS	**
Crude protein	3.9	3.9	4.1	4.0	0.06	NS	NS	*
Crude fat	0.8	0.7	0.7	0.7	0.01	***	NS	NS
NDF[c]	8.4	7.5	7.9	7.7	0.11	***	NS	NS
iNDF[d]	1.6	1.2	1.2	1.1	0.02	***	***	NS
AIA[e]	0.3	0.2	0.2	0.2	0.00	***	NS	NS
WSC[f]	0.4	0.5	0.4	0.4	0.01	***	***	*
Starch	3.7	4.6	4.9	4.6	0.06	***	NS	***
ME[g] (MJ/d)	251	259	270	258	3.5	NS	NS	*
AAT[h] (g/d)	1982	2065	2151	2064	28.1	*	NS	*
PBV[i] (g/d)	703	632	668	677	10.6	***	**	NS
Number of observations	12	12	12	12				
Digestibility, g/kg DM								
Dry matter	714	747	759	740	5.9	***	NS	*
Organic matter	733	764	774	754	5.8	***	NS	*
NDF	659	675	698	690	8.9	NS	NS	NS
Crude protein	712	728	734	690	10.1	NS	**	*
Starch	944	966	974	971	3.8	***	NS	NS

[a]O/B=oats/barley, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, SEM=standard error of mean calculated as n=20, [b]Contrasts were as follows: Difference between the grains (O/B vs. DW), a linear (L) response to supplementing dried wheat for SW in the diet (L=DW vs. SW) and a quadratic response to supplementing dried wheat for SW (Q=DW and SW vs. SW/DW); *P<0.05; **P<0.01; ***P<0.001, [c]Neutral detergent fibre, [d]Indigestible neutral detergent fibre determined using nylon bag incubation technique, [e]Acid insoluble ash, [f]Water soluble carbohydrates, [g]Metabolizable energy, [h]Amino acids absorbed in the small intestine, [i]Protein balance in the rumen.

The analysis of faeces (Table 5) revealed similar results with previous studies. By substituting all of the dry wheat for NaOH –treated wheat, CP content increased (P_L=0.0002) in faeces, but concentrations of starch and NDF decreased (P_L=0.01 and P_L=0.02). The apparent digestibility of starch was more than 94% for all diets. A part of the starch flowed to the small intestine may have been digested in the large intestine. This increased production of endogenous protein in the large intestine and thus declined apparent digestibility of CP, resulting in increased faecal protein output. Consequently, the concentration of urine nitrogen decreased (P_L=0.0003, Table 6). According to these results, the digestion of starch was likely partly shifted from the rumen to the small intestine. ME balance was positive for all diets (two negative values in the data of 82 values). Excretion of faecal nitrogen increased with increasing NaOH –treated wheat in the diet (P_L=0.01). Excretion of urine nitrogen increased as half of the dried wheat was supplemented for NaOH –treated wheat (P_Q=0.002, Table 7). The urine nitrogen excretion was as the lowest in the SW diet. Calculated urine output volume increased by NaOH –treatment (P_L=0.005; P_Q=0.009).

Table 5. Least square means for chemical composition of faeces (g/kg DM).

	Diets[a]					Significance of the contrasts[b]		
Item	O/B	DW	SW/DW	SW	SEM	O/B vs. DW	L	Q
Number of observations	12	12	12	12				
Faecal crude protein	182	197	203	219	3.7	**	***	NS
Faecal starch	34.3	28.7	23.5	22.9	2.76	NS	*	NS
Faecal NDF[c]	467	457	444	432	7.2	NS	*	NS
Faecal AIA[d]	46.4	43.6	45.7	44.1	1.07	NS	NS	NS

[a]O/B=oats/barley, DW=Dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, SEM=standard error of mean calculated as n=20, [b]Contrasts were as follows: Difference between the grains (O/B vs. DW), a linear (L) response to supplementing dried wheat for SW in the diet (L=DW vs. SW) and a quadratic response to supplementing dried wheat for SW (Q=DW and SW vs. SW/DW); *P<0.05; **P<0.01; ***P<0.001, [c]Neutral detergent fibre, [d]Acid insoluble ash.

DMD and OMD increased (P_Q=0.03) as half of dry wheat was substituted for NaOH –treated wheat. Crude protein digestibility (CPD) increased slightly (P_Q=0.04) as half of dry wheat was substituted for NaOH –treated wheat. On the contrary, CPD declined clearly (P_L=0.01) as all dry wheat was substituted for NaOH –treated wheat. No significant difference between treatments was found in digestibility of NDF and starch.

Table 6. Least square means for excretion of nitrogen in urine.

Item	Diets[a]					Significance of contrasts[b]		
	O/B	DW	SW/DW	SW	SEM	O/B vs. DW	L	Q
Number of observations	12	12	12	12				
Urine nitrogen g/L	11.5	11.2	9.1	8.3	0.52	NS	***	NS
Urine output volume L/d	22.5	23.9	34.3	32.0	1.96	NS	**	**

[a]O/B=oats/barley, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, SEM=standard error of mean calculated as n=20, [b]Contrasts were as follows: Difference between the grains (O/B vs. DW), a linear (L) response to supplementing dried wheat for SW in the diet (L=DW vs. SW) and a quadratic response to supplementing dried wheat for SW (Q=DW and SW vs. SW/DW); *P<0.05; **P<0.01; ***P<0.001.

Table 7. Least square means for nitrogen utilization.

Item	Diets[a]					Significance of contrasts[b]		
	O/B	DW	SW/DW	SW	SEM	O/B vs. DW	L	Q
Number of observations	12	12	12	12				
Nitrogen (N) utilization (g/d)								
Total N intake	623	627	662	648	10.1	NS	NS	*
Faeces N	180	170	174	198	7.0	NS	**	NS
N digested	114	117	117	110	10.1	NS	**	*
Urine N	226	259	286	254	7.6	NS	NS	**
Milk N	153	153	152	148	2.8	NS	NS	NS
N balance[c]	34.8	44.5	49.8	48.2	3.98	NS	NS	NS

[a]O/B=oats/barley, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, SEM=standard error of mean calculated as n=20, [b]Contrasts were as follows: Difference between the grains (O/B vs. DW), a linear (L) response to supplementing dried wheat for SW in the diet (L=DW vs. SW) and a quadratic response to supplementing dried wheat for SW (Q=DW and SW vs. SW/DW); *P<0.05; **P<0.01; ***P<0.001, [c]N balance calculated taking into account the body nitrogen retention.

3.3. Milk yield and composition

The composition of milk is shown in Table 8. There was no significant difference between the treatments in milk yield or ECM. Milk fat yield increased slightly (P_Q=0.04) but concentration of milk fat increased clearly (P_Q=0.004) as half of the dry wheat was substituted for NaOH –treated wheat. By substituting all dry wheat for NaOH -treated wheat, milk protein concentration decreased (P_L=0.0001). The same occurred for milk urea concentration (P_L=0.002).

Milk urea content reflects the dietary nitrogen balance in the animal. Low milk urea content indicates, in general, a deficiency of rumen degradable protein. Milk protein content decreased linearly (P_L<0.0001) with increasing NaOH –treated wheat in the diet. In contrary, faecal CP content increased linearly (P_L=0.0002; Table 5). Animals were healthy throughout the experiment. FE value declined as half of the dry wheat was substituted for NaOH –treated wheat (P_Q=0.004).

Table 8. Least square means for milk yield.

	Diets[a]					Significance of the contrasts[b]		
Item	O/B	DW	SW/DW	SW	SEM	O/B vs. DW	L	Q
Number of observations	20	20	21	21				
Production kg/d								
Milk yield	25.8	25.3	25.1	25.4	0.43	NS	NS	NS
ECM[c]	28.6	27.8	28.4	27.6	0.52	NS	NS	NS
Yield of milk fat	1.2	1.1	1.2	1.2	0.03	NS	NS	*
Yield of milk protein	1.0	1.0	0.9	0.9	0.02	NS	NS	NS
Yield of milk lactose	1.2	1.2	1.2	1.2	0.02	NS	NS	NS
Milk composition g/kg								
Fat	47.0	45.8	48.7	46.0	0.79	NS	NS	**
Protein	38.0	39.1	38.1	37.6	0.20	***	***	NS
Lactose	46.2	46.0	46.0	45.9	0.20	NS	NS	NS
Urea	49.5	45.4	42.3	39.0[e]	1.31	*	**	NS
Efficiency								
FE[d]	1.4	1.4	1.3	1.3	0.03	NS	NS	**

[a]O/B=oats/barley, DW=dried wheat, SW/DW=a mix of NaOH-treated wheat and dried wheat, SW=NaOH-treated wheat, SEM=standard error of mean calculated as n=20, [b]Contrasts were as follows: Difference between the grains (O/B vs. DW), a linear (L) response to supplementing dried wheat for SW in the diet (L=DW vs. SW) and a quadratic response to supplementing dried wheat for SW (Q=DW and SW vs. SW/DW); *P<0.05; **P<0.01; ***P<0.001, [c]Energy corrected milk calculated according to Baevre et al. (1988), [d]Feed efficiency (kg ECM/kg DMI), [e]One missing sample, n=20.

3.4. Body weight

The mean values were 629 (SD 66), 626 (SD 71), 626 (SD 69) and 628 (SD 78) kg for O/B, DW, SW/DW and SW diets, respectively. The calculated BW changes was -48.1 (SD 14.25), 45.7 (6.37), 55.8 (SD 1.84), -15.55 (SD 13.76) kg for the diets. In general, increased BW reflects increased body tissue retention but in this study the difference between the treatments was not significant. The DMI calculated as a percentage of the BW was 3.4, 3.4, 3.5 and 3.4 for the experimental diets, respectively.

4 DISCUSSION

4.1. Composition of feeds and TMR

NaOH –treatment increased content of NDF in wheat but decreased that of iNDF in agreement of Mcniven et al. (1995). The neutral detergent washable fraction also likely decreased as suggested by De Campeneere et al. (2006). Hence, a part of starch and protein were compound with NDF. This affects starch degradation and may have prevented ruminal disorders that are connected to excessive decrease in pH. The equal difference in concentrations of NDF and iNDF can be noticed in TMR diets, as well as in the chemical composition of feed ingredients, with increasing the amount of NaOH –treated wheat in the diet. Also decreased starch content in wheat can be noticed in TMR diets as well as in the feed ingredients. Braman and Abe (1977), Mcniven et al. (1995), O'Mara et al. (1997) and Dehghan-Banadaky et al. (2008) have noticed that NaOH-treatment increases the ash content of grains. Ash content increased due to addition of NaOH. The concentrations of other nutrients are reduced because of ash dilution (Mcniven et al. 1995).

The concentration of starch in wheat decreased about 5% by NaOH –treatment. However, the starch content stabilized in diets and no difference was detected between the wheat diets. The ratio of wheat was in this study 340 g DM per kg feed, while in the study of De Campeneere et al. (2006) it was 260 and in the study of Phipps et al. (2001) it was 170. Thus, we had relatively high proportion of wheat in the diets. Hetta et al. (2010) reported positive responses on DMD, feed intake, milk yield and milk protein yield with increasing pelleted wheat content up to 32% wheat of diets. This resulted in a relatively high concentration of ME in wheat diets (12.0-12.2 MJ/kg DM).

4.2. Intake and digestibility

Many feed characteristics affect DMI including the availability of feed, the DM, fibre and energy content and the pH in silage, particle size, characteristics and the concentration of fat, protein and starch in the rumen. The digestibility of feeds has notable implications for energy- and protein-values in feeds, as well as the potential of feed intake. Thus, it also has influence on milk production of dairy cows. Due to higher CF, NDF and iNDF content of oat-barley than wheat resulted in higher intakes of these nutrients in oat-barley diet compared to dried wheat. Starch intake was lower in oat-barley diet than in dried wheat because oat-barley diet is naturally lower in starch.

The hypothesis was that intake of feed increases when the proportion of NaOH –treated wheat increases in the diet. This was based on the high digestibility of feed and on the assumption that the deficiency of energy or nitrogen is not limiting microbial function. Thus, the rate of degradation and passage rate increase, so that the animal should be capable to eat more and fill its nutrient requirements. According to this study DMI and OMI increased as half of the dry wheat was substituted for NaOH –treated wheat. The present study support the suggestion that increased DMD and OMD affect their intakes. Poor silage quality had most likely some effect on intake and especially in SW diet, in which the degradation of seed coat perhaps was not complete or at least DMD was the lowest. The treatment did not respond significant differences in NDFD. Intake of iNDF decreased in the SW/DW and SW diets because the content of iNDF had already decreased by the treatment.

Phipps et al. (2001) reported decreased degradability of DM and starch of wheat in the rumen. According to Mcniven et al. (1995) starch brake-down products were not absorbed enzymatically postruminally although they were digested to sugars. Kung et al. (1983) reported an increase in faecal α-linked glucose. However, in the present study, NaOH –treatment had no effect on starch digestibility. Decreased starch excretion in faeces by NaOH –treatment indicated that starch digestion was shifted from the rumen to small intestine. Assuming that starch degradation in the rumen was reduced, this study suggests that post-ruminal starch digestion occurred to some extent because apparent starch digestibility was numerically greater in SW and SW/DW than in DW diet.

The NaOH –treatment reduced the apparent CPD as all dried wheat was substituted for NaOH –treated wheat. Also in the study of De Campeneere et al. (2006) that happened and according to their rumen incubation study ruminal degradation of starch and CP were reduced by NaOH –treatment. Thus, they concluded that CPD was not completely compensated by digestion in the small intestine. In the present study, faecal CP excretion increased as all dried wheat was substituted for NaOH –treated wheat. The apparent CPD may be reduced partly because of the decrease in ruminal CP degradation. Still the main reason for increased faecal CP content is increased microbial fermentation in the large intestine. Due to the change in starch digestion site, the microbes had more energy available for protein synthesis in the large intestine. The microbes used partly RUP, partly urea from bloodstream as source of nitrogen. Consequently, urinary nitrogen content decreased and faecal nitrogen excretion increased. This theory is consistent with the result reported by Reynolds et al. (2001).

Calculated urine output volume increased significantly by NaOH –treatment. The difference between SW/DW and pure wheat diets was more than 10 liters urine per day. This has impact on use of sludge and may cause need for building a larger sludge pool.

The increase of NaOH –treated wheat in the diet had no effect on BW. The diets affected similar body retention of protein. According to Sriskandarajah et al. (1980) BW increased by feeding cows with NaOH –treated barley compared to rolled barley. Still, their study is relatively old and milk production was low, so that the result is not quite comparable to today. The increased milk fat content in the present study indicates also that body fat was not needed to be mobilized and in this regard the result can be considered relevant.

4.3. Milk yield and composition

Milk protein concentration was decreased due to NaOH –treatment of wheat grains, which may be related to increased microbial fermentation in the large intestine. Because dietary content of CP was high, 180 g/kg DM, it is not likely that there was deficiency of degradable CP in the rumen. There was neither deficiency of energy in the rumen because NaOH –treatment did not decrease feed intake. Otherwise, depress in milk protein content could have been generated from the reduced gluconeogenesis from propionic acid in the liver. The decrease in milk urea content derived possibly from the increased uptake of urea from bloodstream by microbes in the large intestine. However, in general, large intestinal fermentation is difficult to determine and is often based on assumptions. Although NaOH –treatment did decrease milk protein content in wheat diets, it still remained at the same level with control diet.

Acetic and butyric acids are important precursors of short and medium chains fatty acids in the synthesis of milk fat. Mayne and Doherty (1996) reported increased molar proportion of acetic acid in the rumen by NaOH –treatment. However, Sriskandarajah et al. (1980) and Mcniven et al. (1995) did not find any effect on VFA production or composition. An increase in milk fat concentration may be a consequence of increased production of acetic and butyric acids in the rumen. Milk fat content increased slightly as in the study of De Campeneere et al. (2006) but is in contrast to most studies.

Using FE value for economical evaluation showed clearly that animals ate more than they produced milk. This kind of result combined with the decrease in milk protein concentration is clearly uneconomical. In addition, NaOH –treatment is of relatively high cost compared to rolling of grain.

5 CONCLUSIONS

NaOH –treatment alters the chemical composition of wheat grain: concentration of NDF increases and the concentration of iNDF decreases. Increase in NDF concentration arises likely from the chemical reactions. Both protein and starch were bind to NDF, in a manner that it did not separate in the neutral detergent wash. The NaOH –treatment did not result in any economic improvement in this study because concentration of milk protein decreased and intake increased, while milk production remained the same. The best production response to NaOH –treated wheat was achieved with SW/DW diet, in this study. Poor quality of silage had influence on the composition and intake of TMR. Milk production was greater in conventional O/B diet that any of the wheat diets. This suggests that there is no benefit to use NaOH –treated wheat compared with O/B diet.

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