Volatile Fatty Acid Production in Ruminants

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ABSTRACT

Volatile fatty acids (VFA) are important products of ruminal fermentation. The VFA are not only the major source of energy to the ruminant animals but also influence methane production in the rumen. Therefore it is important to understand mechanism controlling VFA production and to depict VFA production in a model. This will allow us to devise strategies to enhance energy utilization and reduce methane production in ruminant livestock. An evaluation of a mechanistic model in predicting VFA production was conducted and equations were introduced into the model to improve the predictions. Later a continuous culture experiment was conducted to test the hypothesis on which those equations were based on.

A mechanistic model – Molly, was evaluated using a dataset with reported VFA production rates. The results of residual error analysis indicated that the root mean square prediction errors (RMSPE) were 63, 63, and 49% for acetate, propionate and butyrate, respectively. An assessment from two studies reporting VFA production revealed a potential of reducing errors of prediction by representing interconversion among VFA. In the second study, equations based on thermodynamics influence of pH and VFA concentration were introduced in the model to represent interconversion among VFA. The parameters for *de novo* VFA production and VFA absorption were re derived with (VFAInt) and without (BASE) the new interconversion equations. There were some improvements in the VFA concentration predictions but the improvements were both in VFAInt and BASE models. The RMSPE of VFA production were still above 50% for acetate, propionate and butyrate. The larger errors of predictions were attributed to measurement variation in VFA production literature, or possible incorrect rate constants for interconversion equations.

Finally, a third study was conducted to assess the effect of pH, and VFA concentration on VFA and methane production in continuous culture. The treatments consisted of control, 20 mmol/d acetate infusion (INFAC), 7 mmol/d propionate infusion (INFPR), and low pH (LOWPH). Individual isotopes of acetate, propionate and butyrate were infused in the fermenters to estimate interconversions among VFA. With LOWPH treatment, methane emission was reduced whereas production of propionate was increased. Hydrogen production was higher in INFAC indicating that some of the acetate could have been degraded to CO_2 and H_2. It was estimated that around 3 % of *de novo* acetate was converted to propionate and 9 % to butyrate. Exchange between propionate and butyrate was insignificant and below 1% of *de novo* production of either VFA. However, treatments did not affect interconversion rates among VFA. These results indicated that pH and VFA concentration do not have thermodynamic influence on VFA interconversion as hypothesized.

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ATTRIBUTION

Some of the chapters included in this dissertation are coauthored. Below is the list of those chapters and the contributions from the co-authors.

Chapter 3. Evaluation of predictions of volatile fatty acid production rates by the Molly cow model. Coauthors: P. Gregorini, and M. D. Hanigan

Thanks to my advisor, Dr. Mark Hanigan, for his guidance in writing and analyzing the data. Dr. Pablo Gregorini, a Senior Scientist at DairyNZ, contributed by editing the paper.

Chapter 4. Representing interconversions among volatile fatty acids in the Molly cow model. Coauthors: R. Kohn, P. Gregorini, and M. D. Hanigan

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Chapter 5. Effects of acetate, propionate and pH on volatile fatty acids and methane production in continuous culture. Coauthors: B. A. Wenner, B. Gill, R. A. Kohn, J. L. Firkins, and M. D. Hanigan

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CHAPTER 1 INTRODUCTION

Ruminant livestock have distinctive ability to utilize structural polysaccharides, not utilizable by humans, and convert them to food products that we consume. It is made possible in large part due to the enzymes secreted by microbes present inside the rumen. Most of the carbohydrate fractions and some of the proteins in the diet of ruminants are fermented by these microbes to volatile fatty acids (VFA), mainly acetate, propionate and butyrate, with the production of carbon dioxide and methane as byproducts. Methane released from ruminants is of environmental concern because of its greenhouse gas potential. Methane is mostly either eructed or exhaled by the animal whereas VFA, unlike glucose and maltose that are absorbed by the small intestine in human, are mostly absorbed in the rumen. These VFA help meet the energy demand of the animal and also affect milk composition and ruminal health. It is therefore important to understand the production and metabolism of VFA both from the standpoint of energy supply to the animal and methane production.

Mostly, the VFA are measured as concentration and relative proportion in the rumen due to the complexities associated with measuring actual production. However, concentration of VFA does not truly represent the production, since it only reflects the balance between production, interconversion, absorption and outflow of VFA in the rumen at a particular time. After the degradation of substrate into VFA, significant carbon interchange can occur among VFA. Furthermore, the ruminal environment can impact VFA production and clearance from the rumen. Few studies have been conducted with the measurement of actual production. Among the methods used for measuring VFA, the isotope dilution technique with more than one VFA carbon labeled provides better estimations of production and clearance of VFA from the rumen. There is limited information in the literature on the impact of the ruminal environment such as, pH, hydrogen concentration and VFA concentration on VFA production. Availability of data on these variables will enhance our understanding of the VFA production in the rumen.

Understanding the mechanism of VFA production and representing it in a model allows us to assess different feeding strategies in animals in terms of their effect on production and cost, both economic and environmental. Both empirical model and mechanistic model are widely used to represent ruminal fermentation. However given the complexities of the rumen and the effect VFA exerts in the rumen such as pH, absorption, and hydrogen supply, mechanistic models are more advantageous for such assessment.

The overall hypothesis of the studies conducted for this dissertation was that pH and VFA concentration have thermodynamic influence on net VFA production. The objectives were to assess thermodynamic influence of pH and VFA concentration on VFA interconversion with modeling and experimental approach. In the first study the Molly cow model was evaluated in terms of its prediction of VFA production in the rumen (**Chapter 3**). Later new equations were introduced in the model to reflect interconversion among VFA and the stoichiometry coefficients were rederived to reduce the errors in prediction of VFA production, along with VFA concentration and ruminal pH (**Chapter 4**). A continuous culture experiment was conducted with the use of isotope of all three major VFA to assess the effect of VFA concentration and pH on VFA carbon dynamics, and VFA and methane production (**Chapter 5**).

CHAPTER 2 REVIEW OF LITERATURE

Introduction

To meet the growing demand of milk and meat, we will need to increase production from ruminant livestock. At the same time, our production systems have to utilize resources efficiently to make sure that producers get better financial incentive and the production practices are environmentally sustainable. Ruminant digestion is an important variable determining production efficiency, quality of production, and environmental impact. Microbial protein and VFA production in the rumen partially determine the efficiency and composition of products. On the other hand enteric methane, due to its greenhouse gas potential, can contribute to global warming. Methane contributes about 21% of global greenhouse gases (GHG) from anthropogenic sources, making it the second major source after carbon dioxide (Forster et al., 2007). In the US, methane contributes about 10% of the total anthropogenic GHG, out of which around 25% comes from enteric methane (EPA, 2010). From the perspective of carbon footprint of entire supply chain of dairy cattle production in the US, enteric methane is the primary contributor (Thoma et al., 2013).

Ruminant Animal Digestion

In agricultural settings ruminant diet consist of forages and grains which are either fed solely or mixed. Forages of grass family (e.g. corn) are dominant, however those of legumes (e.g. alfalfa) are also important components.

The act of collecting the food or the prehension of food differs among species of ruminants. They are categorized as browsers, mixed feeders, and grazers and are distinguished by different morphology of oral organs adapted during the evolutionary process (Pérez–Barbería and Gordon, 2001). Nevertheless, all of them spend significant amounts of time chewing which includes ruminating the ingested food. In dairy cows, the chewing time can range from 1.2 to 8 h/d depending upon the type of the diet (Yang and Beauchemin, 2009). Chewing, both after ingestion and during ruminating, is an important part in digestion which reduces the surface to volume ratio of feed and affects passage rate and available surface for microbial enzymes (Pérez-Barbería and Gordon, 1998). The authors categorized tooth effectiveness and chewing behavior as the variables that affect chewing both of which are influenced by nature of the feed. The act of chewing also stimulates sublingual and sub maxillary glands to secret saliva (McDougall, 1948). In addition to lubricating feed for ingestion, the salvia of ruminant animals is high in bicarbonate and phosphate buffer and devoid of ptyalin (in contrast to that of human) which helps to buffer the rumen pH and also play a role in nitrogen recycling (McDougall, 1948, Bunting et al., 1989). It is estimated that the amount of saliva secreted by beef and dairy cows can range from 108 to 308 L/d (Erdman, 1988). The act of chewing, rumination and their interaction with microbial digestion and passage is an important area to be explored and quantified, and has been largely overlooked (Murphy, 2013).

The amount of food ingested by animals is one of the most important factors affecting productive performance. Various theories have been purposed on how ruminants regulate intake both in short term and in long term (Poppi et al., 1994, Romney and Gill, 2000). From the perspective of production, the goal is to enhance intake together with digestion of the feed for the animals to be efficient by diluting the maintenance cost of production (Bauman et al., 1985). The ingested feed, water and saliva enter into the reticulo-rumen (used synonymously as rumen hereafter) and are acted upon by a host of microbial organisms which include bacteria, protozoa, archaea and fungi whose diversity can vary depending on the diet (Li et al., 2009, Callaway et al., 2010). A conducive environment of pH 5.5 to 6.7, temperature of 39°C, and anaerobic condition allows the microbes to ferment most of the carbohydrate fraction of feed for their own ATP generation and utilize the nitrogenous fraction for protein synthesis (Janssen, 2010). These processes also lead to the production of VFA, ammonia, CO_2 and H_2. Compounds such as formate, ethanol, lactate and succinate are also produced but in smaller fractions (Bauchop and Mountfort, 1981, Krause and Oetzel, 2005). While acetate, propionate and butyrate remain the major VFA contributing about 97% of the total, some branched chain VFA such as isobutyrate and valerate are also produced. Much of these VFA are absorbed across the rumen wall and help to meet the energy demand of the animal which is estimated to contribute up to 72% of the gross energy requirement in cattle (Bergman, 1990). Some of the fermentable fraction of the feed which escapes ruminal degradation is broken down in the small intestine to glucose for absorption.

The protein available to ruminant animals can come either from feed protein that is not degradable in the rumen or as rumen degradable protein which is converted to microbial protein and absorbed in the lower gut (NRC, 2001). More than 80 % of the lipid fraction of feed is hydrolyzed and biohydrogenated in the rumen primarily by bacteria and passed to the lower gut (Lock et al., 2006).

VFA Production and Metabolism in the Rumen

The presence of VFA as the end product of ruminal fermentation was realized as early as 1880s but it was only later in the 1940s that their relative importance as the source of energy was demonstrated (Bergman, 1990). Acetate, propionate and butyrate are the major VFA produced in the rumen, and the proportion of the VFA is largely affected by the type of the diet and the time of the day. For example, Sutton et al. (2003) reported 4.4, 1.3, and 0.5 mol/kg DMI/d of acetate, propionate and butyrate, respectively in a 60:40 diet (concentrate to forage ratio) whereas the production was 3.9, 2.9, and 0.4 mol/kg DMI/d, respectively in a 90:10 diet. Similarly, in non-lactating Jersey cows Esdale et al. (1968) reported production of 5.6, 1.9, and 1.3 mol/kg DMI/d in all corn silage diet and 5, 1.3, and 0.4 mol/kg DMI/d in all alfalfa hay diet for acetate, propionate and butyrate, respectively. In general, availability of readily fermentable fraction of the diet such as starch favors propionate production whereas availability of fiber in the diet favors acetate and butyrate production in the rumen.

De novo VFA Production

The production figures referred to above are however that of net production of VFA, and the *de novo* production (i.e. production from direct degradation of substrate to VFA) might be different. Owing to the significance of VFA in terms of energy supply and methane production, it is important that we are able distinguish these differences and quantify VFA production more accurately.

The structural polysaccharides in the feed are broken down by extracellular microbial enzymes into oligosaccharides and then monosaccharides to produce pyruvate which is hardly detected in the rumen as it is rapidly fermented to VFA (Leng and Brett, 1966). Proteins in the diet can degrade to amino acids which are deaminated and converted to VFA. Production of VFA from protein can be significant in diets high in rumen degradable proteins whereas VFA production from lipid fraction of the diets is usually low (France and Dijkstra, 2005). Pyruvate is converted to acetate and butyrate with acetyl CoA as a common intermediate, whereas propionate can be produced either by a pathway involving oxaloacetate and succinate or by a pathway that involves acrylate formation (Figure 2-1; Ungerfeld and Kohn, 2006).

VFA Pool Dynamics in the Rumen

After substrate degradation, the *de novo* pool of VFA does not remain constant and can change. The loss from each pool is different and is not constant throughout the day. Different routes of loss from each VFA pool include- interconversions among VFA, absorption through the rumen wall, passage of VFA from the rumen, and VFA carbon incorporation into microbes. Some other routes of VFA loss such as condensation of acetate and propionate to valerate (Gray et al., 1952), and degradation of acetate to H_2 and CO_2 also occur in the rumen but to lesser extent (Loughnan et al., 2014).

VFA Interconversion

Although various models of VFA production do not consider interconversion among VFA, there is sufficient information in the literature demonstrating interconversion among VFA. In sheep Bergman et al. (1965) reported that 61% of butyrate carbon was derived from acetate and 20 % of that of acetate from butyrate. They observed much less carbon exchange of acetate and butyrate with propionate. Sutton et al. (2003), reported that VFA interconversions represented 14 to 17% of acetate net production, 13 to 18% of propionate net production, and 58 to 68% of butyrate net production (Figure 2-3). They observed greater carbon transfer from propionate (as the percentage of *de novo* propionate carbon produced) to acetate in normal diet than in low roughages diet. The conversion of propionate to butyrate was minimal, however, conversion of butyrate to propionate was 31% and 93% of *de novo* butyrate production in normal and high concentrate diets, respectively. Because of the variable interconversion among VFA, failure to consider these interconversions would lead to potential errors in prediction of net VFA production.

Control of VFA Interconversion

Chemical reactions are controlled by kinetics and thermodynamics (Kohn and Boston, 2000). Kinetics is related to the reactivity and tells how fast a reaction proceeds whereas thermodynamics deals with stability of a reaction and the direction that a reaction will proceed. In a complex and diverse microbial environment such as the rumen, various biological reactions are possible with the same substrate and therefore the magnitude and the type of metabolic pathway can thus be determined by thermodynamic control (Kohn et al., 2015).

A reaction from A to B is thermodynamically favorable when the free energy going from A to B decreases i.e., when the ΔG is negative. This reaction will be spontaneous and will release energy in the process. Similarly, zero ΔG indicates an equilibrium condition whereas a positive ΔG is thermodynamically unfavorable. However, ΔG does not tell about the rate of the reaction which is determined by the kinetic rate constant of the reaction.

Changes in the diet of the animal not only modify the available substrates but also the rumen environment such as pH, pH_2 (partial pressure of hydrogen) and VFA concentration which will affect thermodynamic states and thus the metabolic pathways of microbes. Kohn and Boston (2000) estimated that the ΔG when glucose is converted to acetate, propionate and butyrate was -140.2, -144.3, and -178.9, respectively. Such large changes in free energy indicates that glucose production from VFA is unlikely without significant energy consumption and the reaction is favorable to proceed to forward direction rather than the reverse. However, similar ΔG for these reactions also indicates that ΔG of VFA interconversion is close to zero at a fixed ruminal environment and the rate of interconversion should be governed by thermodynamic changes in the rumen (Ungerfeld and Kohn, 2006). For example, the authors calculated that acetate to propionate and acetate to butytrate interconversions were -11.2 and 4.2

kJ/mol, respectively for a high roughage diet and -12.5 and -2.8 kJ/mol for a high concentrate diet with some assumptions on unmeasured reactants and products concentration. Such small changes in free energy make these reactions more susceptible to changes in dietary composition which is also supported by the variable VFA interconversion figures reported by Sutton et al. (2003) on concentrate and mixed diet as discussed in previous section.

Thermodynamic influences of VFA concentration and pH_2 on VFA interconversion has also been shown to occur in experiments designed to decipher effects of thermodynamics on anaerobic microbial fermentation. For example hydrogen partial pressure and acetate concentration has been shown to affect butyrate degradation to acetate in anaerobic co-culture with thermophilic butyrate degrading bacteria (Ahring and Westermann, 1988). Moreover, hydrogen utilization for methanogenesis is thermodynamically favorable at higher pH_2 but high pH_2 has also been shown to decrease butyrate and propionate oxidation (Zinder, 1993). For these processes to occur simultaneously in the rumen, the pH_2 must remain in a very narrow range (Ellis et al., 2008). Since it is difficult to get data on H_2 production on various dietary composition whereas VFA concentration and ruminal pH, which also influence VFA interconversion and are affected by diets, are measured regularly in experiments reporting VFA production and interconversions. Therefore, equations based on thermodynamics can be derived for VFA interconversion by using the available data and be used in a mechanistic model framework.

VFA Absorption

Three different mechanism of VFA absorption from the rumen wall have been proposed - 1. Passive diffusion 2. Bicarbonate dependent absorption and 3. Bicarbonate independent absorption. The earlier assumption was that VFA absorption is largely through passive diffusion with only undissociated acids able to permeate the lipid bilayer in the ruminal epithelium (Dijkstra et al., 1993). However, the pK_a of acetic acid, propionic acid and butyric is 4.76, 4.87 and 4.82 respectively, indicating that most of the VFA in normal ruminal pH of 5.8 to 6.5 would be in dissociated form and the VFA absorption will be a lot slower than observed if passive diffusion was the only mechanism. Nevertheless, passive diffusion can occur due to localized pH of epithelial cells near the transporting layer (Dijkstra et al., 2012). The additional proton for this localized epithelial pH can come from sodium hydrogen exchangers exporting protons from the blood into the lumen (Gabel et al., 2002). It is also important to note that passive diffusion does not result in net proton removal from the rumen. The passive diffusion has been estimated to contribute about 28 to 60% of acetate absorption and 69 to 74% of butyrate absorption (Penner et al., 2009). Due to the lipophilic nature of butyrate, the rate of absorption of butyrate from passive diffusion should be higher than acetate but was not observed in an animal experiment possibly due to non-passive transport (Aschenbach et al., 2009).

The absorption through anion exchangers increase the pH in the rumen as bicarbonate along with H can be converted to CO_2 and water by carbonic anhydrase (Gabel et al., 2002). The bicarbonate dependent transport of acetate, which is less lipophilic, has been shown to increase with concentration of VFA in the rumen (Aschenbach et al., 2009).

Similarly, bicarbonate independent and protein mediated transport of VFA can also contribute to net VFA absorption from the rumen (Aschenbach et al., 2009, Penner et al., 2009). However, the exact proportion of all major VFA absorbed from each of these routes has not been well defined yet.

There is contradictory evidence on how the rate of absorption in the rumen epithelium increases in response to high VFA concentration. Bannink et al. (2008) suggested that the elevated absorption rate of VFA in response to VFA concentration in a highly fermented diet can be explained by morphological adaptation in the rumen associated with increased surface area of the epithelium. However, Schurmann et al. (2014) did not find any changes in surface area, rather increased Na and VFA absorption together with increased mannitol flux, tissue conductance and expression of IL-β and TLR2 indicating decreased barrier function in the rumen. Similarly, Etschmann et al. (2009) also observed high Na transport in epithelium of hay and concentrate fed sheep compared to those fed hay only which was sustained up to 12 weeks, and peaked at 4 weeks, most of the increase was at the first week after the diet change. They suggested that increase in surface area of the epithelium when animals are switched to a highly fermented diet is thought to be a delayed response and a more imminent adaptation includes functional changes in existing cells of the rumen epithelium. However, the latter two findings are based on Ussing instrument experiment after serial slaughter of lambs (Etschmann et al., 2009) and young calves (Schurmann et al., 2014) switched on a highly fermentable diet, whereas Bannink et al. (2008) interpretations are based on the observation of cows during early lactation which is characterized by marked hormonal changes. It could be possible that early lactation cows are capable of secreting more GI hormones to enhance the growth of rumen papillae.

Passage of VFA from the Rumen

Most of the VFA produced in the rumen is absorbed through the ruminal epithelium (Bergman, 1990) but few carbons of VFA may pass along with the ruminal fluid or as microbial dry matter into the abomasum. For example, Kristensen (2001) reported less than 1% of acetate carbon exited in the duodenal fluid of non-lactating cows, but the dry matter in duodenum had 7.6% of that produced in rumen of which microbial amino acid and fatty acid accounted for 2.5 %. In sheep on rye grass and forage oats diets, Weston and Hogan (1968) reported that about

24% of the VFA produced in the rumen passed into lower gut and 19% was absorbed in the abomasum and osmasum. In lactating dairy cows Dijkstra et al. (1993) estimated that passage from the rumen contributed 20-35% of total VFA produced in the rumen. Higher liquid volume in the rumen of the cow and consequently higher liquid passage rate could be the main reason why larger proportion of VFA passed from the rumen in lactating cow than in sheep (Dijkstra et al., 1993).

Changes in Microbial Composition

More fiber in the diet increases the diversity of rumen microbes. With high fiber diet the cellulolytic microbes, such as protozoa (+38%), anaerobic fungi (+59%), and methanogens (+27%) population increases compared to that in high starch diet (Belanche et al., 2012). This was also associated with higher pH and higher molar proportion of acetate to propionate. The same authors reported that reduction in nitrogen content of the diet reduced the total bacteria (-13%), anaerobic fungi (-28%), methanogens (-27%), and protozoa (-19%) when compared to normal protein diet. Moreover, low level of forage in the diet increases the passage rate and can washout slow growing microbes such as many methanogens which will not have enough time for higher cell growth and thus overall population (Janssen, 2010).

Even though pH of the extracellular fluid in the rumen is low, the intracellular pH of microbes is at 0.4 to 1 unit higher than that of the fluid pH which is key for their survival and proliferation in the rumen. This is maintained by regulating exchange of H^+ across the microbial membrane, and microbes differ in their ability to withstand low pH by allowing their intracellular pH to drop in response to reduced pH in the fluid (Russell, 1991). Hook et al. (2011) reported that the population of methanogens in the rumen with concentrate diet and hay diet was similar but the population structure and diversity of methanogens were different. The population of archaeal and protozoal communities in the rumen were also not found to be different in TMR based and pasture based diets (de Menezes et al., 2011).

In the bacterial population, *Bacteroidetes* (66.9%) and *Firmicutes* (27.4%) were the dominant bacterial phylum at 45:55 forage to concentrate diets where pH was around 6.46 to 6.51 with various sources of forages (Zhang et al., 2014) . In another study when subacute ruminal acidosis (SARA) was simulated with dietary changes, it was observed that the percentage of *Firmicutes* was increased from 47.23 to 56.85%, and that of *Bacteroidetes* decreased from 44.38 to 34.23% when compared to control. Major reduction of *Bacteroidetes* was observed in *Prevotella* on genus level (17.18% in control vs 9.99% when SARA was induced). However, this reduction of *Prevotella* can be attributed to higher starch diet rather than low pH. Khafipour et al. (2009) induced SARA with both alfalfa pellet and high starch diet and found that animals which were induced SARA with alfalfa pellet diet did not show signs of inflammation with higher population of *Prevotella spp*. specially *P. albensis* which have been shown to produce succinate and propionate as major end products.

Rumen Environment

Ruminal pH

The normal range of pH in the rumen is 5.7 to 6.7 which is less than the general extracellular pH around 7.4. Lower pH in the rumen is because of fermentation of the feed to organic acids which are readily dissociated and can cause decline in pH (Dijkstra et al., 2012).

Type of the diet affects pH of the rumen not only due to change in VFA production pattern but also due to effects on rumination and saliva secretion. For example, high forage to concentrate ratio in the diet will increase rumination time which will stimulate more saliva production thus buffering the rumen fluid (Allen, 1997). When the length of the fiber is longer there is more floating mat in the rumen resulting greater ruminal contraction which can increase absorptive area thus affects ruminal pH (Yang and Beauchemin, 2007). Higher intake coupled with higher fiber intake will also increase the fractional rate of liquid passage rate from the rumen (Dijkstra et al., 2012). However, pH can also affect the pattern of VFA production independent of the diet since at lower pH a greater fraction of soluble carbohydrates are fermented to propionate than at higher pH (Bannink et al., 2008, Calsamiglia et al., 2008). Low pH is also favorable for VFA absorption because more undissociated VFA can be absorbed through passive diffusion on the same absorptive surface of rumen epithelium (Dijkstra et al., 1993).

Maintaining a healthy pH in the rumen is critical particularly for animals on high grain diet since there can be sharp decline in ruminal pH due to highly fermentable feed which affects microbial composition, value of the fermentation products as well as ruminal health. When pH falls below 5.5, ruminal epithelial tissue is damaged contributing to reduced intake and productivity (Krause and Oetzel, 2006). If pH is chronically low it can also lead to diarrhea, liver abscesses, inflammation, and lameness (Plaizier et al., 2008). Reduced pH (<6.0) also inhibits fiber digestion which reduces diet digestibility, absorbed energy supply, animal production, and animal efficiency (Plaizier et al., 2008). For example Plaizier et al. (2001) reported about 20% decrease in 24 h NDF degradation in cows induced with sub-acute ruminal acidosis (SARA). However, Krajcarski-Hunt et al. (2002) observed depressed in situ NDF digestibility only in corn silage and not in grass hay and legume hay when incubated in SARA induced lactating cows. In a continuous culture Calsamiglia et al. (2007) observed that low pH depressed NDF digestibility within the range of pH of 4.9 to 7 independent of the diet type but the depression was marked particularly below 6. Grant and Weidner (1992) also suggested that critical pH for a lag in NDF digestion can range from 6.2 to 5.8 depending upon the type of forages.

It is important to note that the pH of rumen is not uniform and can vary by location and time after feeding. The cranial dorsal part of the rumen has higher pH compared to central and ventral rumen (Shen et al., 2012). Storm and Kristensen (2010) observed around 0.6 unit difference in pH between ventral sac (pH=6.6) and medial mat (pH=6.0) in the rumen of lactating cow fed mixed diet. This variation was not affected by particle size of the forages and ws attributed to increased concentration of VFA in the medial mat compared to ventral sac of the rumen. Difference in pH at different location in the rumen could be due to imperfect mixing of rumen contents and buoyancy of solid particles and the associated microbes therein. The pH of the rumen does not remain the same throughout the day particularly for diets containing highly fermentable fraction of feed (Wales et al., 2009). In a mixed diet or high concentrate diet fed once a day, the pH in the rumen starts to decline immediately after feeding and reaches a nadir at 9 to 11 h and starts to increase again (Hünerberg et al., 2015). Increasing the amount of forages in the diet reduces the diurnal variation in pH in the rumen. A single pH value for various treatments reported in literature for cows which are not fed continuously are generally the average of samples taken at various time of the day after feeding when fermentation is most active. To minimize the bias of rumen location in the reported ruminal pH, researchers usually collect ruminal samples from various locations and measure pH of pooled sample. However in manual sampling, CO_2 in the rumen fluid might escape due to exposure to the ambient air and can result higher reading compared to the actual in rumen (Smith, 1941). Use of rumen loggers in recent years have made continuous measurement of rumen pH possible with good accuracy without exposing the fluid to air (Penner et al., 2006, Hünerberg et al., 2015), but many of these loggers have limited movement in the rumen and generally reside on the reticulum hence cannot give representative pH of entire rumen.

Hydrogen Concentration

Hydrogen is a non-polar gas with poor solubility in water. However, because of the partial pressure of the rumen head space hydrogen, concentrations of hydrogen in the rumen fluid are estimated to range from 90 µM to 250 µM (Hegarty and Gerdes, 1999). Partial pressure of hydrogen is an important factor affecting stoichiometry of VFA production in the rumen (Janssen, 2010). An increase in partial pressure of headspace gas resulted in proportional increase in methane production in a culture experiment when different amount of gaseous hydrogen was introduced into the headspace (Czerkawski et al., 1972). High partial pressure of headspace hydrogen will result in increased hydrogen concentration in the rumen fluid which suppresses NADH ferrodoxin oxidoreductase activity required for continuous oxidation of reducing equivalents and production of hydrogen (Hegarty and Gerdes, 1999). Thus, the diurnal methane production pattern follows that of headspace hydrogen emission as the methanogens rely on hydrogen supply to produce methane. Amount of hydrogen that escapes from the fluid phase is basically that escapes methanogenesis or any other hydrogen sinks. Though hydrogen trapped in microbes is not a significant in amount (Martin, 1998), it is important to realize that the concentration of H_2 and H^+ in extracellular rumen fluids can differ from what is inside the microbes because of differences in membrane permeability (Hegarty and Gerdes, 1999).

Rumen Modifiers

Rumen modifiers have long been used both on farm and experimental settings for modulating the fermentative processes in the rumen to achieve greater efficiency in nutrient utilization. Most of these modifiers can be categorized as ionophores, direct fed microbials, bioactive phytochemicals, and microbial inhibitors.

Monensin is the most widely used ionophores in beef and, recently, in dairy cattle production (Hristov et al., 2013). It is an amonocarboxylic polyether produced naturally by *Streptomyces cinnamonensis*. The effect of monensin in improving the efficiency of energy and protein utilization is well documented (Dinius et al., 1976, Bergen and Bates, 1984, Ramanzin et al., 1997). These effects are attributed mainly to altered VFA production pattern, DM intake, methane production, protein utilization, and feed passage (Schelling, 1984) which occurs mainly due to inhibitory effect on gram positive bacteria (McGuffey et al., 2001). However, consumers' concern over using non therapeutic antibiotics in ruminant production, for the fear of residuals in the meat and milk and development of resistance, has led efforts to explore different strategies to alter rumen environment (Calsamiglia et al., 2007, Benchaar et al., 2008a). In fact the European Union banned its use since 2006 (Chaves et al., 2008).

Among direct fed microbial products, yeast based products are the most widely used rumen modifiers. Supplementation of dairy cow diet with live yeast (*Sacchromyces cerevisiae*) increased NDF digestibility from to 30% to 42% and total tract OM digestibility from 62% to 67%, compared to control (Marden et al., 2008). These increases in digestibility were attributed to reduced redox potential and lower lactate concentration. Yeast has also been shown to accelerate rumen development in young ruminant by stimulating cellulolytic bacteria and protozoa (Chaucheyras-Durand and Fonty, 2002). Improved performance of ruminants with yeast supplementation can be attributed to oxygen scavenging effect of yeast in the rumen (Jouany, 2006). Yeast are aerobic organisms and feed on oxygen trapped in feed particles or those produced by amylolytic bacteria which then creates a favorable micro environment for cellulolytic anaerobes to degrade feed particles (Jouany, 2006). However, the effect of yeast supplementation on improved digestibility is not always elucidated in experiments. For example, Bitencourt et al. (2011) did not find clear improvement in fiber digestibility and DM intake in dairy cows. It is believed that, the strain and supplementation level of yeast used will determine the scale of effects to ruminants and it also has to be supplied continuously to reach a threshold concentration to be effective.

Various bioactive phytochemicals such as essential oils, saponins, tanins, and their active ingredients have been tested over the years in terms of their potential to alter rumen fermentation and for increasing efficiency and decreasing environmental impact (Benchaar et al., 2008a, Hristov et al., 2013). While the makeup of the essential oils chiefly determines its efficacy in increasing nutrient efficiency, it has been proposed that in general essential oils confer positive effect by reducing the protein and starch degradation in the rumen and inhibiting hyper ammonia producing bacteria (Hart et al., 2008). Similarly condensed tannins have been shown to reduce methane and increase propionate concentration has been attributed to its defaunating action (Patra et al., 2010). Many of the phytochemicals tested in culture conditions confer positive influence in ruminal fermentative processes but in actual animal experiments the effects are minimal and or short lived because of the adaptation by the ruminal microbes (Busquet et al., 2006, Benchaar et al., 2008b, Chaves et al., 2008, Hristov et al., 2008).

VFA and Methane Production

Methane is a greenhouse gas with 25 times more global warming potential than carbon dioxide over the 100 year time horizon (Forster et al., 2007). However, the atmospheric life time of methane is only 12 years compared to carbon dioxide which can stay in the atmosphere for several thousand years. It means that reducing methane in the atmosphere will give quick results since if we produce less methane for the next 12 years all the methane that is now in the atmosphere will be lost and its proportion will be low. Therefore, it is being targeted worldwide for reduction. Out of the total human related factors which contribute to the methane emission in the atmosphere, 25% comes from enteric methane in the US (EPA, 2010).

Broadly, methane is produced in the rumen by methanogenic archaea by combining hydrogen and carbon dioxide. There is net gain of CO_2 and release of hydrogen when acetate and butyrate are produced but propionate production does not result net CO_2 production and consumes reducing equivalents depleting source of hydrogen for methanogens (Figure 2-1; Ungerfeld and Kohn, 2006). Therefore, higher propionate production for the same amount of digestible DM results in less methane emission from ruminants (Iqbal et al., 2008). So, it is clear that stoichiometry pattern of VFA not only affects energy supply to the animals but also methane emission.

Archaea in rumen are obligate anaerobes which constitute up to 13.3 % of 16S and 18S rRNA in the rumen and most of these archaea are methanogens (Janssen and Kirs, 2008). Some of the methanogens are also capable of breaking down acetate to methane and CO_2 but most of them utilize H_2 as energy source to reduce CO_2 to CH_4 (Janssen and Kirs, 2008). Although there are recent reports showing presence of methylotropic methanogens in the rumen which do not require hydrogen as energy source (Poulsen et al., 2013), hydrogen has been proposed to play a central role in ruminal fermentation and thus methane production (Ungerfeld and Kohn, 2006, Janssen, 2010). Therefore, even with the relatively smaller biomass the methanogenic archaea play crucial role in disposing the hydrogen and maintaining a favorable environment for VFA production (Janssen and Kirs, 2008).

Strategies to Reduce Enteric Methane Production

Methane mitigation strategies can be divided into three distinct approaches. Firstly, by limiting the production of reducing equivalent of hydrogen. Secondly, enteric methane can be reduced by eliminating methanogens or blocking their pathway for methane production which has been used with various degrees of success. A third approach, which appears to be promising, is - identifying and utilizing alternative sinks of hydrogen in the rumen.

On gross energy basis, including more concentrate or digestible legume forages decreases the amount methane produced in the rumen when compared to grass forages (Kurihara et al., 1999). These diets are readily fermented and result in more propionate production. Shifting VFA stoichiometry more towards propionate will consume more reducing equivalent and reduce hydrogen and thus methane production (Ungerfeld and Kohn, 2006).

The major methanogen in the rumen *Methanobrevibacter ruminantium* has various genes that encode surface adhesion protein (Buddle et al., 2011) that allows them to attach on protozoa in addition to epithelial lining and feed particles (Janssen and Kirs, 2008). Since the methanogens and protozoa symbiosis can contribute up to 37% of methanogensis in the rumen, defaunation has been shown to reduce methane production at varying degrees (Hegarty, 1999). However, the effect of defaunation on reducing methane production does not sustain for long time and it can contribute to reduced fiber digestibility and DM intake (Machmüller et al., 2003, Ranilla et al., 2004, Hristov et al., 2013). Various chemical compounds such as bromocholoromethane, 2-bromo-ethane sulfonate, choloroform and cyclodextrin as well as phtytochemicals have been used successfully to inhibit methanogens (Hristov et al., 2013). Additionally, immunizing animals against methanogens has also been experimented with less clear results (Wright et al., 2004, McAllister and Newbold, 2008, Williams et al., 2009). Most of these effects of hindering the methanogen population are short lived as methanogens are capable of several adaptive mechanisms that would revert their population to the original levels over time (McAllister and Newbold, 2008). Moreover, hydrogen has to be removed from the rumen to prevent accumulation of reducing equivalent of hydrogen that would slow down the process of fermentation.

Using alternative sinks of hydrogen can provide a safer way to reduce methane production and remove hydrogen from the rumen. Reductive acetogenesis, reduction of fumarate and conversion to pyruvate, biohydrogenation, nitrate conversion to ammonia, and sulfate can all act as hydrogen sink in the rumen at varying degrees. Fonty et al. (2007) showed that when gnotobiotic lamb were inoculated with microbiota devoid of methanogens, they had higher acetogenic microbiota population and reductive acetogenesis contributed up to 21% of the fermentation however in the normal rumen it was insignificant indicating that methanogens tend to suppress acetogens in the rumen. However, growing animals in such aseptic condition is not practically possible in farm settings. Slightest exposure to external environment would revert methanogen population back in the rumen eliminating acetogenesis since methanogenesis is thermodynamically more favorable than acetogeneiss (ΔG=-67.4 kJ/mol Vs ΔG=-8.8 kJ/mol; Ungerfeld and Kohn, 2006). Similarly, bio hydrogenation of fatty acids also acts as an insignificant sink of hydrogen contributing to only about 2% of hydrogen in the rumen (Mills et al., 2001). Of the potential dietary approaches, feeding nitrate and sulfate at low levels has apparent merit. Addition of hydrogen to nitrate resulting in ammonia is a thermodynamically more favorable reaction (ΔG=-519 kJ) than conversion of carbon dioxide to methane (ΔG=-134 kJ; Ungerfeld and Kohn, 2006). An added benefit of feeding nitrate is that it generates ammonia which is required by ruminal microbes for protein synthesis and fiber digestion. However, nitrate feeding is limited by the potential risk of nitrate poisoning (methemoglobimia) if nitrite accumulates in the rumen and passes into blood (Leng, 2008). One approach to reduce the risk is to supplement sulfur which promotes conversion of nitrite to ammonia (Leng, 2008). Since sulfur can also accept hydrogen, it can further reduce methane production (Takahashi et al., 1998). Nitrate supplementation in sheep (2.1% of DM) and cow (2.6% of DM) with gradual adaptation has been shown to reduce the methane production (van Zijderveld et al., 2010, Van Zijderveld et al., 2011). However in cows higher methemoglobin was detected in the blood probably due to higher dose (3.4 g of nitrate/d/kg of $BW^{0.75}$ in cows vs 1.6 g of nitrate/d/kg of $BW^{0.75}$ in sheep) in terms of metabolic BW (Van Zijderveld et al., 2011). Therefore, it is critical that proper dose and adaptation period is clearly defined before this approach can be safely introduced in farm level.

Finally, there are evidences of methane oxidation in anaerobic environments. In marine sediments about 80% of the total methane produced is oxidized with sulfate as terminal electron acceptor (Nauhaus et al., 2002). Oxidation of methane by sulfate reduction in ruminal microbials has also been found but it represented only 0.2 and 0.5% of total methane produced (Kajikawa et al., 2003). The authors suggested that it could be because the process of oxidation of methane is slow compared to rumen fermentation.

Methods of Measuring VFA Production

Mostly, VFA are reported as concentration and proportion as a measure for ruminal fermentation characteristic. This is because actual production is difficult to measure due to the dynamics of VFA pool as described in earlier section. Broadly, methods of measuring VFA production can be categorized as tracer based methods and non-tracer based methods.

Non-tracer Methods

Some of the widely used the non-tracer approaches include zero time *in vitro*, perturbation of the steady state, and portal arterial difference methods. In zero time *in vitro* method ruminal contents are sampled from the animal after feeding and incubated anaerobically. The concentration of VFA is measured at different time points, and the declining rate of production is used to estimate the production rate at zero time-indicative of the VFA production rate in the rumen when the samples were taken (Corley Iii and Murphy, 2004). In arterial portal difference method the blood VFA concentration and flow is measured in portal and arterial blood and VFA production is calculated by difference. However, there is significant amount of VFA metabolism in ruminal epithelium particularly of butyrate which can lead to underestimation of the production (Kristensen et al., 2000). In another non-tracer method, when the rumen reaches steady state increasing level of known concentration of a VFA is continuously infused to achieve new steady state conditions. The y intercept of the regression line, infused VFA (mol/d) vs VFA concentration (mM), is indicative of the concentration of VFA for control treatments and the x intercept is the production rate (mol/d) for control treatments. Martin et al. (2001) infused propionate for this approach. The assumption of this approach is that the k or the slope is not altered by the acid infusion which might not be true. High propionate can decrease pH, and affect the rate of absorption and thus production rate prediction could be biased. Moreover, the thermodynamic state of increased concentration of product can make substrate fermentation more favorable towards another product, so increased concentration of propionate can alter acetate to propionate ratio. One of the major drawbacks of non-tracer based methods is that they are not able to measure *de novo* VFA production and interconversion.

Tracer Based Methods

Tracer based methods are used either with radioactive isotope or with stable isotope of VFA giving similar results (France and Dijkstra, 2005). Due to regulatory compliance and safety issues and the associated costs, radioactive tracers are generally not used in animal experiments in recent years. Although hydrogen labeled VFA have been used, because of larger difference in molecular weight of labeled vs unlabeled hydrogen, isotopic discrimination by microbes can occur making the labeled hydrogen more labile thus their reliability is much lower than carbon labeled VFA (Leng and Leonard, 1965). Two approaches are used while using isotope dilution techniques, using only one labeled VFA or mixture of VFA and estimating combined fluxes of other VFA, or separately using individually labeled VFA. In the first method total VFA is assumed as single homogenous pool and rate of VFA production is estimated from specific activity/enrichment and relative proportion of VFA in the samples (Weller et al., 1967). In the second method, each major VFA such as acetate, propionate and butyrate is considered as different compartments and any exchange among them are accounted (Sutton et al., 2003). Because of the variable interconversion among VFA, and lack of clearly defined absorption rates, the first method is not generally used (Hegarty and Nolan, 2007). Therefore the isotope dilution technique with all major VFA isotope provides the most complete assessment of VFA production and clearance from the rumen.

Culture experiments can also be used to study VFA production, interconversion and their determining variables. Fewer amount of isotopes can be used in culture experiments and it is also easier to capture enrichment or specific activities in various pools including microbes and gas. Batch culture experiments, such as those based on the Tilley Terry system (Tilley and Terry, 1963), have been used to assess gas production and forage digestibility. A semi continuous culture system described by Czerkawski et al. (1972) is also widely used where feed is kept in the nylon bag and changed periodically. However, both of these systems have short comings such as inability to continuously sample fluid, measure and control pH, and lack of separate solid and liquid removal. Hoover et al. (1976) described a continuous culture system capable differentially separating solid and liquid effluent to mimic solid and liquid passage rate observed in the rumen. In the dual continuous culture system the contents are homogenously mixed, and the solids are separated as overflow of digested feed (controlled by buffer infusion and liquid removal) whereas the liquid from the fluid is pumped through a filter. The system also has the provision for sampling fluid, controlling pH, and ports that can be used for continuously measuring gas production. It was observed that the CP, amino acid, and true organic matter digestibilities in dual continuous culture system were similar to that in the rumen (Hannah et al., 1986).

VFA Prediction in the Molly Cow Model

Distinct fractions of feed such as starch, cellulose, and hemicellulose differ in their ability to ferment into different VFA. Therefore, to represent VFA production and concentration different stoichiometry coefficients of fermentation from these distinct substrates have been derived (Koong et al., 1975, Murphy et al., 1982, Argyle and Baldwin, 1988, Dijkstra et al., 1992, Bannink et al., 1997). These models are primarily based on those described by Murphy et al. (1982) which was derived after Koong et al. (1975).

Koong et al. (1975) were the first to model the product of fermentation based on dietary composition. In the model, the carbohydrate (starch, non-starch soluble carbohydrate, hemicellulose, cellulose) and protein fractions of the diet is fermented to carbon dioxide, methane, VFA viz. acetate, propionate, butyrate, and valerate and microbial mass. They set arbitrary parameters and, due to the lack of data on VFA production and the inputs for the model, reestimated them by minimizing the errors term calculated after comparing the model output to an experimental data. The basis of their model was as follows.

$$Microbe_{Growth} = \sum_{i=1}^{n} \left(\frac{\left(1-\alpha\right) \times Nutrient_i \times ATP_i}{Y_{ATP}} \right)$$

$$\left\{ \begin{matrix} Acetate \\ Propionate \\ Butyrate \\ \vdots \end{matrix} \right\} = \left\{ \begin{matrix} \sum_{i=1}^{n} \alpha \times Nutrient_i \times A_i \left(2\,acetate + 2H^{+} + 4H_2 + 2CO_2 - 2H_2O \right) \\ \sum_{i=1}^{n} \alpha \times Nutrient_i \times B_i \left(2\,propionate + 2H^{+} + 2H_2O - 2H_2 \right) \\ \sum_{i=1}^{n} \alpha \times Nutrient_i \times C_i \left(butyrate + H^{+} + 2H_2 + 2CO_2 \right) \\ \vdots \end{matrix} \right\}$$

where α, A, B, C, and Y_{ATP} were derived for each nutrient class (i=starch, non-starch soluble carbohydrate, cellulose, and hemicellulose,) by fitting to observed microbial growth and VFA production data given known inputs of ruminally available nutrients. The model was altered by Murphy et al. (1982) with the assumption that observed molar concentration of VFA were equal to the molar proportion of synthezied VFA. This also allowed them to use large set of data mainly of beef and sheep consisting of 60 different diets based on roughages (>50% of DM) and 48 diets on concentrate based diets with reported VFA concentration. The results were two separate set of coefficients for *A*, *B*, and *C*, one each for forage and concentrate based diet (Figure 2-2).

The Molly cow model is a dynamic mechanistic model in which VFA prediction is based on those described by Murphy et al. (1982). Using a mechanistic model has distinct advantages over using empirical models because the rumen is a complex system and representing in a mechanistic framework will allow to quantify hydrogen balance as well as assessing new strategies for mitigating methane production (Ellis et al., 2010). Additionally, given the diversity of diets in ruminant production systems, mechanistic models have been shown to predict methane production more accurately than simple regression models (Benchaar et al., 1998).

In the original Murphy et al. (1982) model two separate sets of coefficients of conversion, one for high concentrate diet and the other for high forage diet were used. Later Argyle and Baldwin (1988) used an additional mixed set by taking the average from the two sets of coefficients. Using discrete sets of coefficient however might not represent the differences in VFA production pattern observed in the range of diet used in bovine production (Ghimire et al., 2014).

The derivation of stoichiometry of Murphy et al. (1982) was from VFA concentration data rather than production rates and are based on the assumption that the observed molar concentration of VFA were equal to the molar proportion of synthesized VFA. However, concentration is just the balance of *de novo* VFA production from different nutrient fraction of the feed and loss from each pool of VFA from the rumen at a particular time point, and thus may not be indicative of actual production (France and Dijkstra, 2005). For example, when Sutton et al. (2003) analyzed the relationship between VFA concentration and VFA production in dairy cows fed high concentration or normal mixed diet they found that the slopes and intercept of the regression were different for VFA. Only propionate had r^2 of 0.9 and intercept close to zero whereas for acetate and butyrate the r^2 were 0.4 and 0.6 and intercepts of 15.6 and -9.7, respectively. Similarly, Kristensen (2001) estimated that about 28% of gross production of ruminal acetate exits as metabolites other than acetate. Since interconversion exists among VFA, lack of representation could contribute significant bias in prediction. As discussed earlier, it has been shown that the interconversions among VFA are not constant across diets and can be controlled by thermodynamics. Therefore, in a mechanistic model such as Molly, the VFA production model can be improved by implementing equations based on thermodynamics and rederiving the stoichiometric coefficient of Murphy et al. (1982) along with rate constants for VFA absorption. One of the major limitations of applying thermodynamic model for interconversion is that there are not enough data on reactant and products. However, VFA and H^+ concentration, two major variables in the thermodynamic equations (Ungerfeld and Kohn, 2006), have been widely measured on range of diets. Similarly, various studies have quantified VFA interconversions along with their production rates (Esdale et al., 1968, Rogers and Davis, 1982, Sharp et al., 1982, Armentano and Young, 1983, Seal and Parker, 1994, Sutton et al., 2003, 2008, Markantonatos et al., 2009). Assuming constant values for some of the unmeasured variables such as pH_2, pCO_2, and ADP and ATP concentrations (Kohn and Boston, 2000), the rate constant for the thermodynamic equations for interconversion can be calculated and the resultant equations can be used in the Molly cow model for improving the prediction of VFA. Having said that, collection of data on these unmeasured variables in future experiments could be beneficial for devising more robust models.

Since ruminal pH has profound influence on VFA stoichiometry, Argyle and Baldwin (1988) used linear equations to represent the effect which was included in the Molly cow model. They altered the coefficients of acetate and propionate for cellulose, starch and soluble CHO between pH of 5.4 to 6.2 and shifted coefficient entirely for lactate for pH below 5.4. This type of discontinuous linear relationship might not be true and derivation of a sigmoidal curve with the use of VFA dataset containing wide range of pH could better represent influence of pH on VFA stoichiometry (Baldwin, 1995).

The Molly cow model uses Briggs equation to predict ruminal pH from VFA and lactate concentration (Argyle and Baldwin, 1988). Hanigan et al. (2013) found that the model under predicted pH by 0.5 units and modified the intercept and slope to improve the prediction. Moreover, ruminal pH can also affect the rate of VFA absorption from the epithelium by altering the proportion of dissociated to undissociated VFA proportion (Dijkstra et al., 1993, France and Dijkstra, 2005, Bannink et al., 2008). However, this has not yet been represented in the model which could have contributed to errors in VFA concentration prediction and potentially to its production prediction. Similarly, when analyzing the effect of ruminal epithelial blood flow on VFA absorption Storm et al. (2011) found that the net absorption of propionate was correlated (r=0.56) to the epithelial blood flow. The model that they devised could be integrated into the Molly cow model to reflect such effect and improve the absorption rates and overall VFA dynamics in the rumen.

Summary

Ruminal fermentation has the unique ability to degrade complex carbohydrate to VFA which can be utilized by the animals as energy source. The influence that VFA exerts on enteric methane production further underscores the importance of understanding VFA production in the rumen. Although various methane abatement approaches have been tested, we are still far from an effective strategy that can be successfully implemented in wide range of production systems. Because VFA and enteric methane production are interrelated, our pursuit to identify and assess methane mitigation approaches can be limited due to poor understanding of VFA production. Ruminal environment such as pH, hydrogen and VFA concentration can have profound impact on VFA production and clearance in the rumen. Thus our knowledge of VFA production in the rumen could enhance if the effects of these variables on VFA production and pool dynamics are properly quantified and represented.

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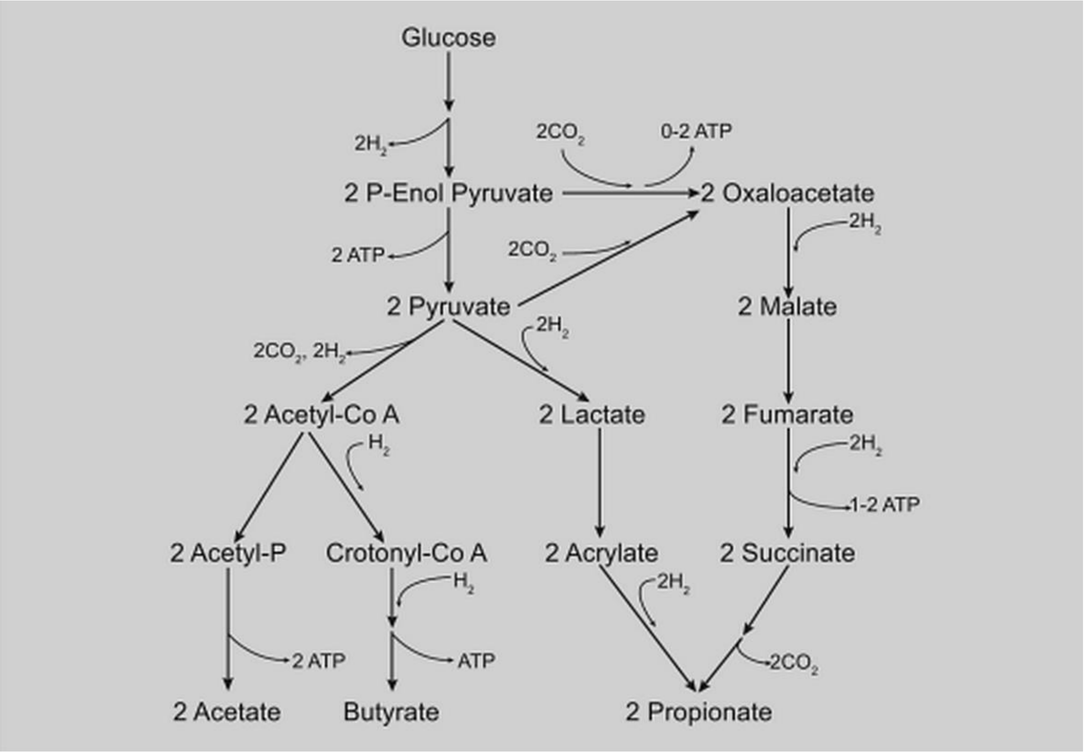

Figure 2-1 Pathway of fermentation in the rumen. H_2 is donated via $NADH^+$ and $FADH_2$. Adapted from Ungerfeld and Kohn (2006).

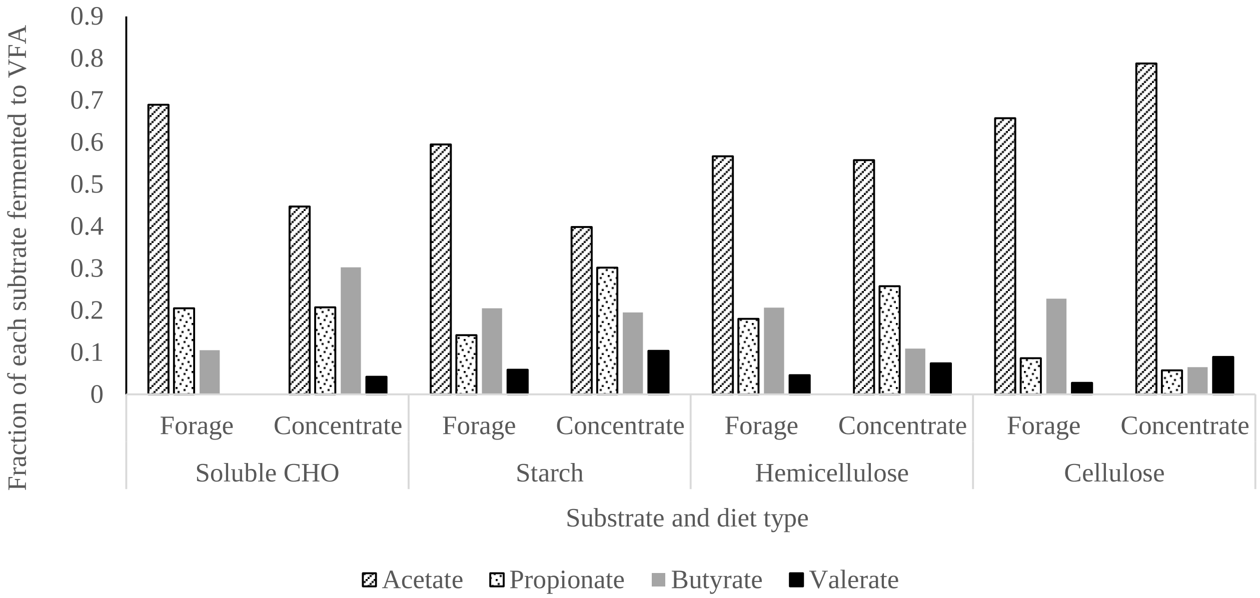

Figure 2-2 Volatile fatty acids (VFA) stoichiometry from different substrate provided in a high forage or high concentrate diet. Adapted from Murphy et al. (1982)

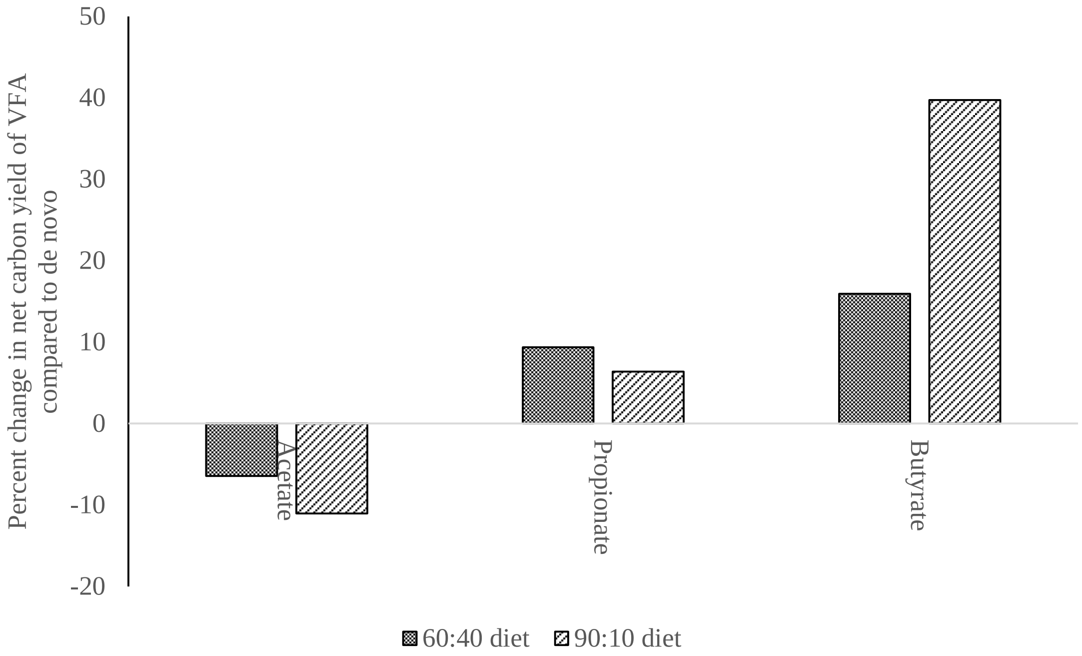

Figure 2-3 Percent change in *de novo* carbon yield of acetate, propionate and butyrate due to after interconversion fluxes in 60:40 and 90:10 concentrate to forage diets. Adapted from Sutton et al. (2003).

CHAPTER 3 EVALUATION OF PREDICTIONS OF VOLATILE FATTY ACID PRODUCTION RATES BY THE MOLLY COW MODEL

Ghimire, S., P. Gregorini, and M. D. Hanigan. 2014. Evaluation of predictions of volatile fatty acid production rates by the Molly cow model. J. Dairy Sci. 97:354–362.

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Abstract

Predicting ruminal volatile fatty acid (VFA) production is important, as they supply energy to the animal and also dictate methane production. The VFA production submodel in the Molly cow model was evaluated using data from eight publications that reported VFA production rates for cattle. Evaluations were conducted with ruminal water balance predictions enabled and the ruminal VFA stoichiometry coefficients set to "mixed" for all diets, or "mixed" when forage represented between 20 and 80% of the diet, "concentrate" when < 20% forage, or "forage" when >80% forage. Prediction errors were relatively insensitive to changes in VFA coefficients by diet type. Root mean square prediction errors (RMSPE) were 63, 63, and 49% for acetate, propionate, and butyrate production rates, respectively. A large proportion of the error was slope bias for acetate and butyrate, and a modest proportion for propionate. Because interconversions between acetate and propionate represent approximately 15% of the variation in net production rates, lack of such consideration in the model may contribute to the substantial model prediction errors. The potential of using thermodynamic equations to predict interconversions was assessed using observed ruminal pH and VFA concentrations from two studies and assuming constant hydrogen pressure and concentrations of CO_2, H_2O, ADP, ATP, and P_i. Rate constants for conversion of acetate to propionate and propionate to acetate were derived independently from the control treatments and used to predict the fluxes for the other treatment. The observed changes in VFA concentrations and pH explained the observed changes in conversion of acetate to propionate, but over predicted the change in propionate to acetate flux in one study. When applied to the other study, the equations predicted the increase in propionate to acetate flux, but failed to predict the observed reduction in acetate to propionate flux. The inability to predict responses accurately may be due to a lack of data for controlling factors other than pH and VFA concentrations.

Introduction

Volatile fatty acids (VFA) are products of enteric microbial fermentation that contribute about 72% of the energy required by the cow (Bergman, 1990). Volatile fatty acid production rates also determine ruminal hydrogen supply which is used for methane production (Elliot and Loosli, 1959). Pyruvate conversion to propionate consumes electrons thereby reducing hydrogen supply (Janssen, 2010), and formation of acetate and butyrate release hydrogen (Kohn and Boston, 2000). Thus ruminal fermentation patterns are linked to methanogenesis. Although there is some diversity in the metabolic pathways used to metabolize fermentable substrate among microbial species, the overall stoichiometry of VFA production is assumed to remain constant (Rodríguez et al., 2006). Thus, if VFA production can be understood and predicted, it will be possible to understand and predict hydrogen supply. Measurements of hydrogen production *in vivo* are difficult and inaccurate, thus mechanistic representations of rumen metabolism can help to understand and assess manipulations of rumen fermentation for reducing hydrogen generation and thereby methane production (McAllister and Newbold, 2008).

Initial efforts to develop predictive models of individual VFA, such as the research work of Murphy et al. (1982), described a set of stoichiometry constants for conversion of each rumen fermented nutrient (starch, non-starch soluble carbohydrate, hemicellulose, cellulose, and protein) to microbial biomass, acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate (net yields) based on the model of Koong et al. (1975). The original model was devised to relate observed microbial growth and VFA production data to known inputs of rumen available nutrients. The challenge that Murphy et al. (1982) encountered was the lack of measured rates of VFA production. To circumvent the problem, a value for the fraction of fermented substrate converted to microbial biomass was assumed and observed ruminal VFA molar concentrations were assumed equivalent to the molar proportions of synthesized VFA. Also, lack of data on minor VFA forced a reduction of the model to acetate, propionate, butyrate, and valerate. The derived stoichiometric coefficients for conversion of each nutrient to these 4 VFA were found to be different between high concentrate and high forage diets, thus a set of coefficients was derived separately for each diet type. Argyle and Baldwin (1988) subsequently adopted these coefficients for use in the Molly cow model and used the average of the forage and concentrate coefficients to create a mixed class yielding three discrete sets of coefficients.

Models based on these stoichiometric coefficients have been evaluated for accuracy and precision in predicting ruminal VFA concentrations (Bannink et al., 1997, Hanigan et al., 2006, Morvay et al., 2011), but little work has been undertaken to evaluate predictions of production rates and absorption rates of individual VFA. Therefore, it is unclear whether the observed bias in predicting VFA concentrations relates to problems in predicting VFA absorption or VFA production. Bannink et al. (1997) explored various hypothesis for the observed prediction errors and also concluded that the cause was either associated with predictions of production or absorption.

Although, based only on concentration data, one cannot rule out the possibility that all of the problems are caused solely by absorption. Bannink et al. (1997) was unable to completely resolve the problem through changes in the representation of absorption. Improvements were realized with acetate, but no progress was made for propionate, butyrate, or valerate predictions. Thus current evidence would suggest that absorption predictions are not the primary cause of the prediction errors, although they may be contributing, thereby leaving production predictions as the primary hypothesis.

The stoichiometry constants of Murphy et al. (1982) are based on the implicit assumption that substrate supply is the primary determinant of production rate, i.e. the reaction series is essentially irreversible or that reversibility is constant across diets and end-product concentrations do not affect net flux rate. However, all the studies that use a fully interchanging model and more than one VFA isotope have reported significant interconversion between VFA which will affect the net amount of individual VFA produced and absorbed by the host animal. For example, Sutton et al. (2003), reported that VFA interconversions represented 14 to 17% of acetate net production, 13 to 18% of propionate net production, and 58 to 68% of butyrate net production. When comparing propionate to acetate conversions for the low forage diet in this study, a condition that resulted in increased propionate production and propionate concentrations, the conversion increased by 50%. Similarly, when Seal and Parker (1994) infused propionate into the rumen, the propionate to acetate conversion increased by 50% and the acetate to propionate conversion decreased by 50% resulting in a fairly substantial shift in net synthesis of each. Failure to consider these variable interconversion rates contribute error to current predictions of net VFA production rates.

Although zero time *in vitro*, perturbation of the steady state, and portal arterial difference methods have been used to estimate net production rates of VFA, they all have drawbacks. Most importantly, they all lack the ability to measure *de novo* synthesis rates and interconversions. Thus the tracer method is currently the only method of estimating *de novo* VFA production rates in the rumen (France and Dijkstra, 2005). Tracer based methods are implemented with the use of radioactive or stable isotopes with similar results. Although hydrogen labeled VFA have been used, their reliability is much lower than carbon labeled VFA (Leng and Leonard, 1965b). Two approaches are used while using isotope dilution techniques, using only one labeled VFA and estimating combined fluxes of other VFA, or using individually labeled VFA. However, use of a single VFA isotope is not generally used because of the variable interconversion between VFA and lack of clearly defined rates of absorption (Hegarty and Nolan, 2007). Therefore, the isotope dilution technique provides the most complete assessment of VFA production and clearance from the rumen.

Since the conversion of pyruvate to acetate, propionate or butyrate share common intermediates and all of the reactions have negative free energy, the flow of carbon between these VFA can be explained by thermodynamic control (Ungerfeld and Kohn, 2006). Thus we hypothesized that the cause of bias in predictions of VFA concentrations in the Molly cow model was inappropriate predictions of VFA production rates. The main objective of the current work was to evaluate predictions of VFA production rates by the Molly cow model using bovine data assembled from studies reported in the literature to determine accuracy and precision. Secondly, this work evaluated the potential of using thermodynamic equations to predict VFA interconversions.

Materials and Methods

Simulations

The Molly cow model described by Baldwin (1995) with modifications (Hanigan et al., 2006, 2009) including recently derived digestion parameters (Hanigan et al, in press) was used. Simulations were conducted with acslX (V3.0, Aegis Technoligies Group, Inc. Huntsville, AL). The model was run for 14 d for each simulation to ensure steady state was achieved, and predictions in the last day of the simulation were used for comparison to published observations. The evaluations were conducted with ruminal water balance predictions enabled. These equations should better predict the change in water volume that occurs on different diet types and thus provide better estimates of concentrations of VFA. Ruminal VFA stoichiometric coefficients of Murphy et al. (1982) as deployed by Argyle and Baldwin (1988) in Molly were set initially for a “mixed” diet regardless of diet composition, and subsequently to “concentrate” when the diet contained less than 20% forage, “forage” when it contained more than 80% forage, and to “mixed” for the remainder to test whether considering diet type had a significant impact on prediction accuracy as originally observed.

Evaluation Data

Observations of VFA production rates and concentrations collected from dairy and growing cattle were assembled from the literature (Esdale et al., 1968, Rogers and Davis, 1982, Sharp et al., 1982, Armentano and Young, 1983, Seal and Parker, 1994, Sutton et al., 2003, Markantonatos et al., 2008, Markantonatos et al., 2009). Esdale et al. (1968) measured VFA production on corn silage and alfalfa hay. Rogers and Davis (1982) had Holstein steers intraruminally infused salts in high grain and high roughages diet. Sharp et al. (1982) used ground and whole grain in steers. Armentano and Young (1983) and Markantonatos et al. (2009) examined the effect of monensin on VFA production whereas in Seal and Parker (1994) study the effect of 3 different levels of propionate infusion was examined. Sutton et al. (2003) and Markantonatos et al. (2008) had high concentrate and low concentrate diet. Only publications using isotope dilution techniques and reporting enough dietary information to generate inputs for the model were used (Table 3-1). The studies varied in terms of location of rumen sampling. Sutton et al. (2003) and Sharp et al. (1982) sampled rumen contents from the ventral rumen, Seal and Parker (Seal and Parker, 1994) provided no information regarding the location of sampling, and rest of the studies sampled from multiple locations and pooled to get a representative sample. Location of rumen sampling affects the pH and VFA concentration along with other parameters such as ammonia and sodium concentration (Shen et al., 2012). Residual errors for pH and VFA concentration and production rates were analyzed to assess the effects of sampling location using the Mixed procedure of SAS. Because diet type (forage, concentrate, or mixed) is also clearly related to these variables and may be an underlying cause for residual errors, it was also included in the model. Residual errors were associated with diet type ($P<0.01$) but were not associated with sampling location ($P=0.29$) suggesting that the effects of location were not large and thus inclusion of studies with divergent sampling location did not significantly bias results. Because all combinations of sampling location and diet type were not present in the dataset, it was not possible to test the effect of their interaction.

Reported DMI and body weight were used as model inputs. Missing nutrient values were predicted from the NRC (2001) feed library except starch solubility and degradation which were predicted from Tamminga et al. (1990) or Miller and Hoover (1998), and ruminal ADF degradability which was assumed to be similar to that of NDF and was predicted from the equation of Huhtanen et al. (2010).

Residual errors of prediction were used to calculate root mean square prediction errors (RMSPE). The RMSPE were expressed as a percentage of the observed treatment means and partitioned into mean bias, slope bias, and dispersion proportions (Bibby and Toutenberg, 1977).

Assessing Potential for Model Improvement

To test the potential of applying thermodynamic regulation of VFA production within the Molly cow model, the following equations of Ungerfeld and Kohn (2006) were used to describe interconversions between acetate and propionate and tested with two studies (Seal and Parker, 1994, Sutton et al., 2003) which contained treatments that affected the thermodynamic balance within the rumen.

$$F_{A,P} = K_{A,P}[Acetate][CO_2]{P_{H_2}}^3([ADP][P_i][H^+])^n$$

$$F_{P,A} = K_{P,A}[Propionate][H_2O]^2([ATP][H_2O])^n$$

$K_{A,P}$ ($L^{(2+3n)}$ $mol^{-(1+3n)}$ atm^{-3} d^{-1}) represented the rate constant for acetate to propionate conversion and $K_{P,A}$ ($L^{(3+2n)}$ $mol^{-(1+2n)}$ d^{-1}) represented the rate constant for propionate to acetate conversion. Each rate constant was derived by study from the control treatment, the normal forage treatment of Sutton et al. (2003) and the saline infusion of Seal and Parker (1994), by algebraic rearrangement of the equations using the observed pH and VFA concentrations and assumed constant hydrogen pressure (P_{H_2}, atm) and concentrations (mol/L) of CO_2, H_2O, ADP, ATP, and P_i (phosphorus) (Ungerfeld and Kohn, 2006). The rate constants derived from the control treatment were then used with the same equation and observed pH and VFA concentrations from the other treatment in each study to predict fluxes between acetate and propionate ($F_{A,P}$ and $F_{P,A}$, mol/d). At equilibrium there could be infinite number of forward and reverse flux combination that will give a ratio of 1, therefore this approach anchored the control treatment in each of the studies to come up with the kinetic rate constants for the reaction. However, it is important to remember that this rate constant may not represent true rate constant because of the assumption of fixed values for some reactants and products of the reaction due to insufficient data in the literature. The efficiency of ATP use (n) was set to 0.6 as derived by Ungerfeld and Kohn (2006).

These equations are based on the principle that the ruminal fermentation reactions are limited more by the free energy change (ΔG) between the substrate and product of a reaction. A reaction from A to B is thermodynamically favorable when there is decrease in free energy going from A to B i.e. when the ΔG is negative. This reaction will be spontaneous and will release energy in the process. Similarly, zero ΔG indicates an equilibrium condition whereas a positive ΔG is thermodynamically unfavorable. However, ΔG does not tell about the rate of the reaction which is determined the kinetic rate constant of the reaction.

The conversion of pyruvate to acetate, propionate, and butyrate have a negative ΔG and are spontaneous, and since they all share common intermediates, the interconversion between these VFA are also thermodynamically regulated (Ungerfeld and Kohn, 2006). At equilibrium the forward and reverse flow between any two VFA should be equal when adjusted for the stiochiometrical coefficient. When a reaction is not in equilibrium the tendency to attain equilibrium will regulate the flow of the reaction.

Results and Discussion

Using only the “mixed” diet VFA coefficients, regardless of dietary forage to concentrate ratios resulted in RMSPE of 20, 41, and 30% for acetate, propionate, and butyrate concentrations, respectively; with small mean bias for propionate and relatively small proportions of slope bias for acetate and butyrate (Table 3-2). The RMSPE for VFA production rates were 63, 63, and 49% for acetate, propionate, and butyrate, respectively. As a reference point, the SEM from Sutton et al. (2003) were 12.2, 11.5, and 19.1% for synthesis of acetate, propionate, and butyrate respectively. Thus the large errors of prediction did not arise solely from variance in observed data. Acetate and butyrate production rate predictions had a large proportion of the error as slope bias and propionate production had a modest proportion as mean bias. Ruminal pH was predicted with a RMSPE of 7% with minor mean bias and very little slope bias. Therefore, the lack of fit for VFA production data was apparently not due to problems with ruminal pH predictions.

Setting VFA stoichiometric coefficients by diet type did not result in improved predictions of acetate and butyrate production with RMSPE of 64 and 49%, respectively, and only a minor reduction in error for propionate (58% RMSPE). There were no major changes in the proportioning of error among mean bias, slope bias, and dispersion (Table 3-3).

The slope bias for production of acetate (Figure 3-1) and butyrate predictions (Figure 3-2) was pronounced representing 35 and 56% of the overall prediction error, respectively (Table 3-3), while little evidence of slope bias was present in propionate predictions (Figure 3-3). The negative slope for acetate production residuals is consistent with a slope less than 1 for regressions of production rate on concentration observed by Nozière et al. (2011) given that the Murphy et al. (1982) coefficients were derived using concentration data. But negative slope bias for butyrate is not consistent with a slope greater than 1 for the production and concentration regression of Nozière et al. (2011). The lack of concordance between production and concentration for acetate and butyrate indicates that production, absorption, or passage from the rumen are being influenced by factors associated with the respective VFA concentrations, any of which undermine the Murphy et al. (1982) assumption that concentration and production are absolutely proportional. Obviously this would have biased the original stoichiometric derivations. However, if that were the only problem, bias in the production predictions and not in the concentration predictions would be expected.

Predictions of acetate and butyrate concentrations also had negative slope bias while there was no slope bias for propionate concentration predictions (Table 3-2). The presence of negative slope bias for acetate and butyrate concentrations and the lack of such bias for propionate suggest to rule out problems with passage assuming all three VFA are passing by bulk liquid flow. These observations, and those of Morvay et al. (2011) clearly indicate that the representation of VFA production is more complex and cannot be achieved with a simple set or even multiple sets of coefficients. If improvements in prediction accuracy are to be achieved, a different and more holistic approach is needed.

A potential contributing factor to the above prediction errors is the assumption that the net yield of individual VFA from a given nutrient is fixed. Because interconversions among VFA occur and the extent is subject to the variable thermodynamic forces in the rumen, the net yield of individual VFA cannot be fixed with respect to nutrients fermented. For example the presence of high proton (H^+) concentrations in the rumen would push net yields from acetate and butyrate to propionate (Hegarty and Gerdes, 1999). When rate constants for interconversion of acetate and propionate were derived from the high forage diet of Sutton et al. (2003), thermodynamic forces predicted the observed increase in acetate to propionate interconversion and propionate to acetate conversion for the low roughage diet. However, the absolute changes in the latter were overpredicted (Table 3-4). The predicted changes in acetate to butyrate exchange were also well predicted by this approach (data not shown). When applied in a similar manner to the propionate infusion data of Seal and Parker (1994), the change in propionate concentration elicited the observed increase in propionate to acetate flux, but the model failed to predict the observed reduction in acetate to propionate flux. InSutton et al. (2003) study the low roughages treatment has a 90:10 ratio for concentrate and hay compared to 60:40 in the control. Similarly, Seal and Parker (1994) infused 1 mol/d of propionic acid. Therefore in both of the studies one could expect one would expect increased flow of propionate to acetate than acetate into propionate based on thermodynamics. The observed and predicted increase was 1.5 and 1.3 times inSutton et al. (2003) and 3.2 and 1.8 times in Seal and Parker (1994) study, respectively. In the latter work, ruminal pH was not reported. A shift in ruminal pH and change in available H or other products associated with the infusion could have explained the observed reduction in acetate to propionate flux. In the same manner, lack of complete data or, perhaps, use of inappropriate exponents for the equations could explain the overprediction of changes in propionate to acetate flux for the Sutton et al. (2003) experiment. Clearly some of the assumed reactant or product concentrations must be wrong as the derived rate constants for the forward and reverse rates should be equal given that the reactions occur through the same pathway.

Despite the limitations in applying thermodynamic concepts to the rumen, the fact that interconversions of VFA occur and that they appear to be thermodynamically driven undermines the approach taken by Murphy et al. (1982) to derive a set of stoichiometric coefficients for net conversion and perhaps explains some of the large prediction errors associated with predictions of VFA production for those equations. The use of VFA concentrations to derive stoichiometric coefficients also adds to the prediction error as the relationships are not proportional across VFA (Nozière et al., 2011). However, the general approach may still have merit if the coefficients are derived for net synthesis rates with consideration of thermodynamically driven interconversions, and if VFA absorption and passage are predicted without bias.

Perspective

It is unclear how well measurements of VFA production from sheep represent those of cattle. Nozière et al. (2011) were able to accommodate sheep and buffalo data in their meta-analyses; however, they exhibit different eating and digestive patterns compared with cattle (Van Soest, 1982) which may limit their use in deriving bovine parameters. Because data used herein were restricted to those generated from cattle, the dietary variation required to develop a robust set of predictions was not possible. However, they may be adequate to anchor predictions of VFA production rates while utilizing the much larger concentration data set to gain the required variation in dietary inputs. Because the small VFA production data set includes observations of VFA concentrations, these data can also be used to correct any problems with VFA absorption predictions in the model. A proven absorption model overcomes the limitation associated with assuming concentrations are proportional to production (Murphy et al., 1982). With confidence in absorption predictions, it then becomes possible to derive power from the extensive observations of VFA concentrations reported in the literature to derive production rates. This would result in enough dietary diversity to ensure robustness of the derived stoichiometric coefficients. If this approach is undertaken, it is critical that the coefficients are derived from a model that considers thermodynamically driven interconversions of VFA so that the new coefficients reflect *de novo* synthesis rates. This combination approach should generate more accurate predictions of net production which will be responsive to diet as well as ruminal reactant and product concentrations.

From the analyses presented above, it is not possible to determine if three discrete sets of coefficients as derived by Argyle and Baldwin (1988) are adequate to represent the full range of dietary conditions required to serve the cattle industry. If one assumes there are 10 microbes that represent the majority of the total biomass and that each has a unique VFA production profile, then as many as 10 sets of coefficients may be required. Nagorcka et al. (2000) used this approach, assuming three species, and achieved more accurate predictions of VFA concentrations in the Dijkstra et al. (1992) model. However, the improvement may simply reflect a better set of coefficients, as the number of coefficient sets used is the same as used by Argyle and Baldwin (1988), and thus no additional complexity was added to the model. The approach simply substituted three diet types by three sets of microbes.

Including a thermodynamic submodel should allow exploration and prediction of a broader range of methane mitigation strategies than currently possible. Modifiers of rumen function that affect passage rate or absorption rate such as buffers, salt, feeding frequency, etc. will impact ruminal reactant and product concentrations. Some of these may result in significant changes in VFA patterns. Unique combinations of such strategies that result in significant shifts in VFA patterns may not be evident, but could be identified with such a model. The model would also allow quantitative relationships relating inputs and responses to be developed which allow rationale economic decisions to be made.

Conclusions

The Molly cow model did not perform well in predicting ruminal VFA production rates as evidences by RMSPE of 50% or more for three major VFA. Variation in VFA interconversion appear to be driven at least partially by thermodynamic forces which results in a significant violation of one of the assumptions used by Murphy et al. (1987) to derive the original estimates of net synthesis rates of VFA as used in the model. Although data that can be used to define thermodynamic state in the rumen are minimal, it may be possible to improve predictions by including themodynamic equations for interconversion of VFA with just VFA and H^+ concentrations as driving variables and refitting VFA fermentation coefficients. If the approach proves beneficial, it will likely stimulate collection of the additional data in future experiments.

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Table 3-1 Summary of the reported data used to evaluate predictions of ruminal volatile fatty acids (VFA) production. Source data were treatment means from the experiments of Armentano and Young (1983), Esdale et al. (1968), Markantonatos et al. (2008, 2009), Rogers and Davis (1982), Seal and Parker (1994), Sharp et al. (1982), and Sutton et al. (2003).

Variable	**N**	**Mean**	**SD**	**Minimum**	**Maximum**
DMI, kg/d	23	10.14	5.00	3.50	19.60
DMI(g/kg BW)[1]	23	24.53	15.02	12.83	67.20
Body weight, kg	23	479	198	127	661
Acetate, mol/d	23	23.06	12.54	6.64	56.50
Propionate, mol/d	23	10.87	8.35	1.90	40.10
Butyrate, mol/d	21	6.15	2.17	1.55	10.30
Total VFA, mmol/L	18	112.29	24.00	84.90	156.00
Acetate, mmol/L	18	63.36	13.10	46.50	92.60
Propionate, mmol/L	18	32.18	11.85	15.70	68.94
Butyrate, mmol/L	18	13.33	3.87	8.40	25.33
VFA production rate (mmol/kg BW/d)					
Acetate	23	53.47	30.72	18.92	135.17
Propionate	23	24.06	14.82	6.51	61.69
Butyrate	21	12.96	6.17	5.31	31.72
VFA production rate (mol/kg DMI/d)					
Acetate	23	2.80	1.43	0.72	5.60
Propionate	23	1.24	0.82	0.40	3.16

Butyrate	21	0.70	0.36	0.23	1.66
pH	18	6.29	0.44	5.37	6.85
Roughage (% of DM)	23	53.19	31.03	10.00	100.00
CP (% of DM)	23	13.92	2.17	8.80	17.20
Fat (% of DM)	23	3.16	0.95	2.00	5.20
Starch (% of DM)	23	31.80	21.03	0.00	62.56
NDF (% of DM)	23	33.82	13.05	15.30	57.70
ADF (%of DM)	23	21.94	9.87	8.40	36.90
Lignin (%of DM)	23	3.46	1.41	1.52	5.77
Ash (%of DM)	23	5.98	2.40	3.12	10.00
Soluble CP (% of CP)	23	36.66	6.00	25.60	51.30
RUP (% of CP)	23	23.98	8.00	11.99	44.30
NPN CP (% of total CP)[2]	23	1.75	4.56	0.00	15.59
Urea CP (% of total CP)	23	1.75	4.56	0.00	15.59
Soluble Starch (% of starch)	23	32.86	14.53	0.00	64.50
Undegraded Starch (% of starch)	23	40.77	15.70	0.00	51.26
Rumen Undegraded ADF (% of ADF)	23	54.75	7.542	38.14	70.52
Forage NDF (% of NDF)	23	67.13	32.11	0	100

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[1] DMI (g/kg BW) for the experiment of Seal and Parker was 67.2 for two treatments. Animals were fed at the rate of 2.8 g of DM/kg BW every hour in 24 equal lots.

[2] including CP from urea.

Table 3-2 Residual error analyses for predictions of ruminal volatile fatty acid (VFA) production and concentrations using the Murphy et al. (1982) VFA submodel within the Molly cow model (Baldwin, 1995) with modifications (Hanigan et al., 2006, 2009). Rumen liquid volume was predicted from osmotic balance. VFA stoichiometric coefficients were set to “mixed”[1] for all simulations.

Variable	N	Mean Observed	Mean Predicted	RMSPE[2]	Mean Bias	Slope Bias	Dispersion
				% of Obs	------ % of MSPE ----		
Acetate synthesis, mol/d	23	23.10	27.20	63	8	33	59
Propionate synthesis, mol/d	23	10.90	13.30	63	12	3	85
Butyrate synthesis, mol/d	21	6.15	6.46	49	1	56	43
Ruminal pH	18	6.29	6.13	7	13	8	79
Volatile fatty acids, mmol/L	18	11.20	10.30	23	13	13	75
Acetate, mmol/L	18	63.0	66.00	20	4	14	82
Propionate, mmol/L	18	32.00	25.00	41	32	0	68
Butyrate, mmol/L	18	13.00	12.00	30	5	12	83

[1] VFA coefficients for mixed diets as defined by Argyle and Baldwin (1988) were used for all diets

[2] Root mean square prediction expressed as the percentage of observed mean.

Table 3-3 Residual error analyses for predictions of ruminal volatile fatty acid (VFA) production and concentrations using the Murphy et al. (1982) VFA submodel within the Molly cow model (Baldwin, 1995) with modifications (Hanigan et al., 2006, 2009). Rumen liquid volume was predicted from osmotic balance. VFA stoichiometric coefficients[1] were set to “forage” for diets with greater than 80% forage, “concentrate” for diets with less than 20% forage, and “mixed” for the remaining diets.

Variable	N	Mean Observed	Mean Predicted	RMSPE[2]	Mean Bias	Slope Bias	Dispersion
				% of Obs	----- % of MSPE -----		
Acetate Synthesis, mol/d	23	23.1	26.9	64	7	35	58
Propionate Synthesis, mol/d	23	10.9	13.6	58	18	1	80
Butyrate Synthesis, mol/d	21	6.15	6.49	49	1	56	42
Ruminal pH	18	6.29	6.13	7	13	6	81
Volatile Fatty Acids, mmol/L	18	11.20	10.30	23	13	13	73
Acetate, mmol/L	18	63.0	65.00	20	1	18	81
Propionate, mmol/L	18	32.00	26.00	36	32	0	68
Butyrate, mmol/L	18	13.00	12.00	29	6	7	86

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[1] VFA coefficients for concentrate, mixed, and forage diets as defined by Argyle and Baldwin (1988) were used as specified.

[2] Root mean square prediction error expressed as the percentage of the observed mean

Table 3-4 Predictions of interconversions (mol/d) between acetate (A) and propionate (P) for 2 studies in the literature (Seal and Parker, 1994, Sutton et al., 2003). The rate constants were calculated from the control data and used to predict the alternative treatment in each study. All substrates were considered in each flux[1].

		Calculated K		Observed Flow (mol/d)		Predicted Flow (mol/d)	
Study	$[H^+]$ (M)	A to P	P to A	A to P	P to A	A to P	P to A
Sutton et al., 2003							
Normal	$1.7x10^{-07}$	$7.6x10^{20}$	0.226	1.2	1.53	1.20	1.53
Low Roughage	$3.2x10^{-07}$			1.45	2.3	1.42	3.21
% Change	90%			21%	50%	18%	109%
Seal and Parker, 1994[2]							
Control	$3.2x10^{-07}$	$1.32x10^{20}$	0.063	0.35	0.41	0.35	0.41
P infusion	$3.2x10^{-07}$			0.2	0.64	0.360	0.653
% Change	0%			-43%	56%	3%	59%

[1] The forward and reverse rate constants were calculated from observed flows for control treatments using the equations of Underfeld and Kohn (2006): $K_{(A\text{->}P)} = V_{(A\text{->}P)} / [CH_3COO^-] [CO_2] PH_2^3([ADP][P_i][H^+])^n$; $K_{(P\text{->}A)} = V_{(P\text{->}A)} / [CH_3CH_2COO^-][H_2O]([ATP][H_2O])^n$, where $K_{(A\text{->}P)}$ = rate constant for the acetate to propionate flux, $K_{(P\text{->}A)}$= rate constant for the propionate to acetate flux, $V_{(A\text{->}P)}$ = velocity for the acetate to propionate flux (mol/d), $V_{(P\text{->}A)}$ = velocity for the propionate to acetate flux (mol/d), [x] = concentration (mol/l), n= mols of ATP generated or consumed. Concentrations of VFA and velocities were as reported. Concentrations of H^+ were determined from pH. CO_2 and H_2 concentrations were determined as previously described (Kohn and Boston, 2000). Concentrations of ADP, ATP, Pi and water were as previously reported (Ungerfeld and Kohn, 2006).[2] H^+ concentrations were assumed for the Seal and Parker (1994) experiment

Figure 3-1 Residual errors versus predicted for predictions of ruminal acetate production using the model of Murphy et al. (1982) as represented in the Molly cow model (Baldwin, 1995) with modifications (Hanigan et al., 2006, 2009).

Figure 3-2 Residual errors versus predicted for predictions of ruminal butyrate production using the model of Murphy et al. (1982) as represented in the Molly cow model (Baldwin, 1995) with modifications (Hanigan et al., 2006, 2009).

Figure 3-3 Residual errors versus predicted for predictions of ruminal propionate production using the model of Murphy et al. (1982) as represented in the Molly cow model (Baldwin, 1995) with modifications (Hanigan et al., 2006, 2009).

Figure 3-1 Ghimire et al.

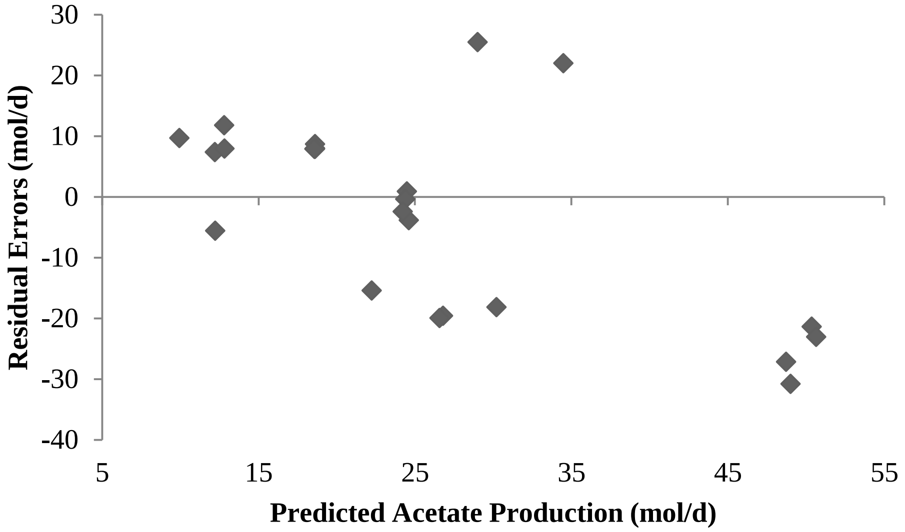


Figure 3-2 Ghimire et al.

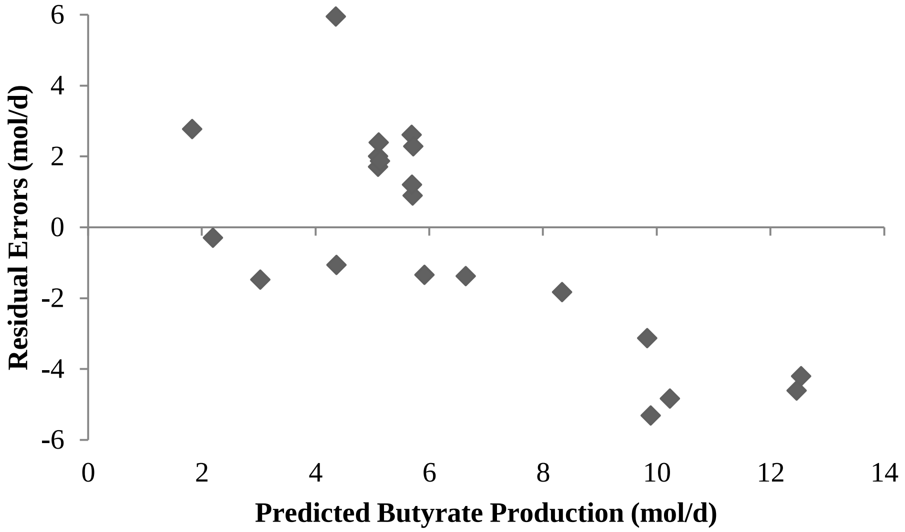
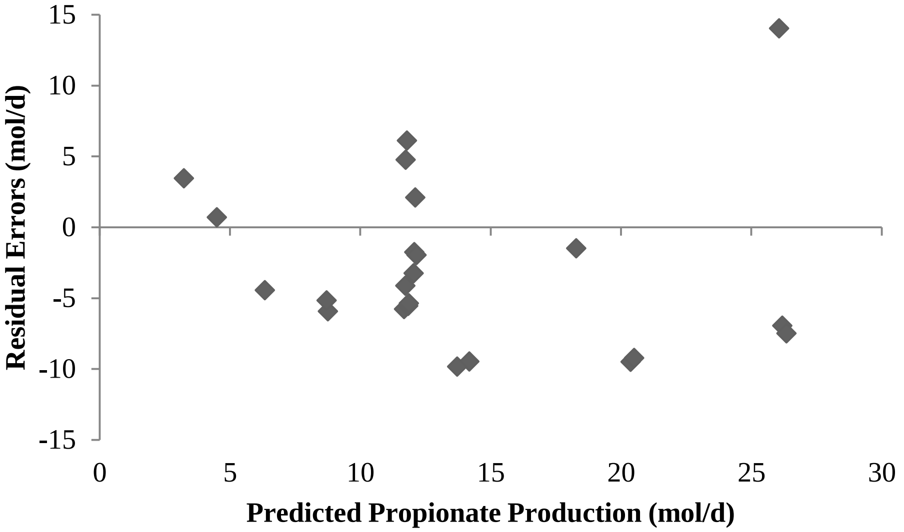


Figure 3-3 Ghimire et al.



CHAPTER 4 REPRESENTING INTERCONVERSIONS AMONG VOLATILE FATTY ACIDS IN THE MOLLY COW MODEL

Abstract

The Molly cow model uses stoichiometric coefficients from individual dietary fractions of forage and concentrate for predicting volatile fatty acid (VFA) production. We previously showed these predictions were associated with large errors and hypothesized that it was due to a lack of representation of carbon exchange among VFA. The objectives of the present study were to add VFA interconversion equations based on thermodynamics to the Molly cow model and evaluate the effect of these additions on model accuracy and precision. Thermodynamic equations described by Ungerfeld and Kohn (2006) were introduced to represent interconversions among VFA. The model was further modified to use a linear interpolation of *de novo* acetate, propionate and butyrate production coefficients from individual dietary nutrient fractions of high forage and high concentrate diets for application to mixed diets based on forage to concentrate ratios. Both the original model and the modified one were reparameterized and evaluations were conducted using 8 studies reporting pH, VFA concentration, and VFA production rates using isotope dilution technique and 62 studies reporting VFA concentration and pH. Evaluations after parameter estimation revealed that predictions of VFA production rates were not improved with root mean squared prediction errors (RMSPE) of 77, 60 and 51% for acetate, propionate and butyrate, respectively in revised model vs 75, 63, and 55, respectively for the original model. The RMSPE for predictions of VFA concentrations were reduced from 28, 46, and 40 to 22, 31, and 26 simply by reparameterizing the existing model, but there were minimal further improvements associated with the addition of thermodynamically driven interconversion equations with predictions having 21, 32, and 27% RMSPE for acetate, propionate and butyrate respectively.

Including the effect of pH on VFA absorption reduced the mean bias of propionate production and slope bias of VFA concentration and acetate production but not the overall RMSPE. The larger prediction errors for VFA production could be due to measurement variation, poor representation of the effects of pH on stoichiometric parameters, or incorrect rate constants for VFA interconversions. Additional data are required to discriminate among these hypotheses.

Introduction

Volatile fatty acid (VFA) production in the rumen contributes to energy supply to the animal, ruminal health, milk composition, and methane production. Acetate, propionate and butyrate are the major VFA produced in the rumen and contribute more than 95% of the total VFA formed. While most of the butyrate is metabolized in the ruminal wall, acetate and propionate are largely transported to the liver (Kristensen et al., 2000). In the liver, propionate acts as a gluconeogenic precursor and is an important source for milk energy output whereas acetate is an important carbon source for peripheral tissue ATP production and for *de novo* fatty acid synthesis (Hanson and Ballard, 1967, Young, 1977). The pattern of VFA production in the rumen also influences hydrogen affecting methane production from methanogens. Therefore accurate predictions of VFA production in the rumen are important in representing ruminal function of nutrient based animal models.

The Molly cow model is a dynamic mechanistic model in which the VFA prediction is based on the stoichiometry coefficient described by Murphy et al. (1982). The coefficients are based on the assumption that substrate supply is a primary determinant of VFA production rates, and interconversions are assumed to either not occur or be constant across diets. However, our recent study demonstrated that these predictions are associated with large errors (Ghimire et al., 2014). The interconversions among VFA has been proposed to be controlled by the thermodynamics of the reactions which is partially driven by ruminal pH and VFA concentrations (Ungerfeld and Kohn, 2006). Furthermore, poor representations of substrate degradation to VFA and lack of representation of the effect of pH on VFA absorption in the model may cause inaccurate estimation of VFA (Dijkstra et al., 1993, Bannink et al., 1997).

We hypothesized that depicting carbon exchange among VFA can improve the prediction of VFA production by the Molly cow model.The objective of this study was to introduce thermodynamically driven VFA interconversion equations into the Molly cow model, refit the coefficients describing VFA synthesis and absorption, and evaluate the impact of the changes on errors of prediction of VFA production rates and concentrations.

Materials and Methods

Simulations

The Molly cow model described by Baldwin (1995) with modifications (Hanigan et al., 2006, 2009, 2013) was used. Simulations were conducted with acslX software (V3.0, Aegis Technologies Group, Inc. Huntsville, AL). The model was set to run for 10 days for each simulation to ensure steady state condition, and the results from the last day were compared to observed values. Murphy et al. (1982) devised VFA stoichiometric coefficients based on the Koong et al. (1975) model and defined the coefficient separately for forage and concentrate based feeds. Argyle and Baldwin (1988) later added a mixed diet set of coefficients which was the average of forage and concentrate coefficient sets and added the scheme to the Molly cow model (Baldwin, 1995). However, using discrete sets of coefficients introduces discontinuities into the model which generally are not well tolerated in optimization problems (Floudas and Pardalos, 2008). Therefore *de novo* acetate, propionate and butyrate production fluxes were calculated with both forage and concentrate coefficients, and linear interpolations based on the forage to concentrate ratio were used to predict the flux:

$$F_i = F_{i,For} \times f_{For} + F_{i,Con} \times (1 - f_{For}) \tag{1}$$

where F_i is the production of VFA (acetate, propionate or butyrate) in mol/d from *i* substrate (cellulose, hemicellulose, starch, or soluble carbohydrate) in mol/d, $F_{i,For}$ is the coefficient of F_i from forage fraction of the diet and f_{For} is the fraction of forage in the diet. For reparameterization these parameters were bounded to ensure complete carbon balance and estimated along with VFA absorption constants.

The following equations as described by Ungerfeld and Kohn (2006) were used to represent carbon interchange among the VFA.

$$F_{A,P} = K_{A,P}[Acetate][CO_2]{P_{H_2}}^3([ADP][P_i][H^+])^n \tag{2}$$

$$F_{P,A} = K_{P,A}[Propionate][H_2O]^2([ATP][H_2O])^n \tag{3}$$

$$F_{A,B} = K_{A,B}[Acetate]^2{P_{H_2}}^2([ADP][P_i][H^+])^n \tag{4}$$

$$F_{B,A} = K_{B,A}[Butyrate][H_2O]^2([ATP][H_2O])^n \tag{5}$$

$$F_{P,B} = K_{P,B}[Propionate][CO_2]{P_{H_2}}^2([ADP][P_i][H^+])^n \tag{6}$$

$$F_{B,P} = K_{B,P}[Butyrate][H_2O]^2([ATP][H_2O])^n \tag{7}$$

where $F_{i,j}$ is the flux of i to j (mol/d), where i and j represented A (acetate), P (propionate), or B (butyrate). Rate constant of respective fluxes are denoted by $K_{i,j}$. Rate constants were calculated from 6 treatment means from the literature used in the dataset which reported pH, VFA interconversion, VFA net production and concentration by rearrangement of the above equations (Equations 2 thorough 7). The concentration of CO_2, H_2O, ADP, ATP, P_i (phosphorus), and partial pressure of hydrogen were assumed constant and the values were used as described by Ungerfeld and Kohn (2006). The efficiency of ATP use (n) was set as 0.6 for $F_{A,P}$ and $F_{PA,}$ 0.47 for $F_{P,B}$ and $F_{B,P}$, and 0.13 for $F_{A,B}$ and $F_{B,A}$.

Because there was bias in residuals of VFA concentration and production on predicted pH after final parameter estimation, following equation as described by Dijkstra et al. (1992) was later introduced in the models used for parameter estimates

$$Abs_i = \frac{V_{max} \times RumVol^{0.75}}{\left(1 + \frac{0.338}{[i]}\right)(1 + \left(\frac{pH}{6.45}\right)^{6.48})} \qquad (8)$$

where Abs_i is the absorption rate of i VFA (mol/d) and i represented acetate, propionate or butyrate. The V_{max} is the maximum potential absorption of VFA (mol/d), $RumVol$ (L) is the ruminal liquid volume, $[i]$ is the concentration of VFA (M), and pH is the ruminal pH. A new V_{max} was derived by fitting model prediction of ruminal pH, VFA concentration, *de novo* and or net production and used for reevaluation.

Dataset for Model Evaluation and Reparameterization

The dataset including VFA production rate described by Ghimire et al. (2014) was combined with the one described by Hanigan et al. (2013) with reported VFA concentration and pH. The later was a subset of those used by the NRC committee to formulate the 2001 nutrient requirement model (NRC, 2001). The final dataset contained 193 treatment means for acetate, propionate and butyrate concentrations and 23, 23 and 21 treatment means for acetate, propionate and butyrate production, respectively.

An evaluation of the model was done before estimating the parameters. Parameter estimations were done in two scenarios, one after introducing equations 2 through 6 (VFAInt model), and the other before introducing the equations (BASE model) to assess if any improvement in the model predictions were due to the newly introduced equations. For parameter estimations, VFA stoichiometry parameters along with the VFA absorption rate constants were estimated by fitting model predictions of ruminal pH, VFA concentration, VFA *de novo* and or net production (synonymously referred to as 'production' in this paper) to observed values using Quasi Newton optimization algorithm in acslX program to maximize log likelihood function (LLF). In the BASE model the *de novo* production was equal to the net production and thus only *de novo* was used for fitting. To ensure carbon balance, parameters for *de novo* VFA production from different substrate for propionate and butyrate were estimated and those for acetate were calculated by difference based on degradation pattern per unit glucose and amino acid to each VFA as described by Baldwin (1995). Minimum and maximum bounds were initially set to 50% of the original parameters and were later increased to 75% until none of the parameters rested on the bound.

Residual errors of prediction were used to calculate root mean squared prediction errors (RMSPE) expressed as percentage of the mean, and RMSPE were partitioned into mean bias, slope bias, and dispersion (Bibby and Toutenberg, 1977). A post hoc analysis of residuals was done by regressing the residuals of VFA production and concentration against the predicted VFA interconversion, and ruminal pH.

Global sensitivity of net production of VFA to the VFA stoichiometry coefficients and absorption rate constants (as listed in Table 4-3) were undertaken. Fourier amplitude sensitivity test provided in acslX software (Ver. 3.1; Aegis Technologies Group, Huntsville, AL) as described by Saltelli et al. (1999) was used for the analysis. In short, the first order sensitivity coefficient of a parameter *X* was calculated as

$$S_i = \frac{Var_X[E(Y|X)]}{Var_Y} \tag{9}$$

where, X is the input parameter, Y is the output variable, $E(Y|X)$ is the expectation of Y which is dependent on the fixed value of X, Var_X is the variance of the sampled parameter values taken from the sapling space, and Var_Y is the variance of the simulated value of the output variable Y. Second order sensitivity indices were calculated after considering the interaction of parameter X with other parameters. And the total effect index of parameter X on output Y was calculated by summing the first order sensitivity index with all second order sensitivity indices. This total effect index of a parameter, designated as global sensitivity coefficient, indicates the fraction of the variation in the model due to the parameter X.

For the global sensitivity analysis in acslX, the sampling boundaries were set to 70% and 130% of final parameter estimates. The limit on the number of parameters for the sensitivity analysis was increased to 25 parameters as described by Schaibly and Shuler (1973). Resampling and interference factor, which determines the number of parameter samples for simulation, was set to 4. These settings resulted in a total sampling population of 792,372 observations from which global sensitivity coefficients were derived.

Results and Discussion

Initial evaluations of the model before fitting revealed that the RMSPE of acetate, propionate and butyrate concentrations were 28, 46, and 40%, respectively, with relatively large mean bias and small proportion of slope bias (Table 4-1). The RMSPE for VFA production were 69, 58 and 50% for acetate propionate and butyrate, respectively, with large mean bias for propionate and large slope bias for acetate and butyrate. These errors of VFA production were slightly larger for acetate and minutely lower for propionate than those reported by Ghimire et al.

(2014) who used a separate set of coefficients according to diet type. This could be because the linear interpolation were done assuming that the initial coefficients of forage and concentrate were for 100% inclusion whereas they were derived for high forage or high concentrate, not 100% inclusion. Therefore, some bias can be expected by simply using initial coefficients with Equation 1 without re deriving coefficients. The error of pH prediction was minimal with some mean bias and very low slope bias.

With equations for VFA interconversions in place in the VFAInt model the RMSPE of acetate, propionate and butyrate concentration were reduced to 21, 32 and 27 %, respectively with the elimination of mean bias after the parameters were re derived. The mean concentration predictions thus were close to the observed data. However, there was a modest slope bias for all VFA (12 to 14%). One possible explanation for the observed error is inaccurate predictions of liquid passage rate in the model which is calculated as constant fractional proportion of rumen liquid volume (Baldwin, 1995). Improvement of prediction of ruminal liquid volume and the liquid passage rate might help reduce the observed prediction errors in VFA concentration. The RMSPE for VFA productions were however not improved and were 77, 60, and 51% for acetate, propionate, and butyrate, respectively, in the VFAInt model after final parameter estimate. This was associated with over prediction of total VFA production by 9 mol/d which is substantial considering that the observed mean production was 40 mol/d. Significant proportion of errors were exhibited by mean bias for propionate production and slope bias for acetate and butyrate production. These results indicate that the improvement in concentration predictions might have been due to improvement in absorption parameters, and the interconversion equations along with rederived parameters were not able to reduce the errors.

In the BASE model the re derivation of parameters resulted in reduction of RMSPE of concentration to 22, 31, and 26% for acetate, propionate and butyrate, respectively. The reduction was due to elimination of mean bias. The RMSPE of acetate, propionate and butyrate production were however not improved (Table 4-1).

Although there were some improvements in prediction of VFA concentration by representing VFA interconversion in the model, the prediction of VFA production did not improve. Furthermore, the improvements in concentration predictions were also observed in the BASE model after reestimating parameters. In fact when the log likelihood function (LLF) after the final parameter estimate was used to perform a Chi-square test, the BASE model had significantly higher LLF ($P < 0.05$) than the VFAInt model. This indicates that improvement in concentration prediction VFAInt could be due to better representation of stoichiometry and absorption coefficient in the model and not due to representations of carbon interchange by the VFAInt model.

A *post hoc* residual analysis revealed that the residuals of acetate concentration had some slopes to predicted NDF and starch (Figure 4-1) in VFAInt model. Propionate and butyrate concentration however did not reveal such slope. Similarly, residuals of VFA production had significant slope ($P = 0.018$ to 0.074) to predicted NDF and starch content in the diets but because of the dispersed residuals the r^2 ranged only 0.16 to 0.26 (Figure 4-2). These slopes were similar in BASE model as well (figure not shown). The residuals of concentration of all VFA had significant slope against predicted pH both in VFAInt (Figure 4-3) and BASE (figure not shown) model with concentration under predicted at high pH and over predicted at lower pH. The slope was also present in propionate production with r^2 of 0.35 (Figure 4-4). This could be because of the lack of representation of effect of pH on VFA absorption in the model.

Absorption of VFA is favored by low pH in the rumen because of increased dissociated form of the VFA whereas at higher pH the absorption rate is lower (France and Dijkstra, 2005). Therefore, at low pH due to higher clearance from absorption the concentration may fall down and lack of representation of this effect results over prediction concentration at lower pH. To represent the effect of pH on VFA absorption, Equation 8 as described by Dijkstra et al. (1992) was introduced by replacing the existing equation based on mass action in both VFAInt and BASE models. The V_{max} was re derived by fitting model predictions of ruminal pH, VFA concentration, *de novo* (only in VFAInt model) and net production to observed values. This fitting resulted in reduction in slope bias of VFA concentration but that did not improve the RMSPE of VFA concentration or production in both VFAInt and BASE model (Table 4-1). Moreover, the mean bias of propionate concentration increased with the absorption adjustment for both models. The proportion of mean bias was reduced for propionate production in the VFAInt model and slope bias for acetate production in both VFAInt and BASE model. That however, did not result significant improvements in predictions of VFA concentration and production with only minimal improvement for acetate production in VFAInt model.

The initial value of the parameters along with the final estimates for both VFAInt and BASE model are presented in Table 4-3. The parameters that were most changed (>50% of original) in the VFAInt model included *KabsAc* (rate constant of acetate absorption) and *KabsPr* (rate constant for propionate absorption) along with *fScBuBufr* (parameter for butyrate production from degradation of soluble carbohydrate fraction of forage), *fStBuBufr* (parameter for butyrate production from degradation of starch fraction of forage), and *fScBuBucn* (parameter for butyrate production from degradation of soluble carbohydrate fraction of concentrate). For *de novo* propionate production those that were most changed (30 to 45%) in the

VFAInt model were *fScPrPrfr* (propionate production from degradation of soluble carbohydrate fraction of forage), *fHcPrPrcn* (propionate production from degradation of hemicellulose fraction of concentrate) and *fCePrPrcn* (propionate production from degradation of cellulose fraction of concentrate). The change in *de novo* acetate production parameters were acetate production from soluble carbohydrate and starch fractions of concentrate (44 and 49% change, respectively). In BASE model the parameters most affected were (50 to 65% change) were *KabsAc*, *KabsPr*, *KabsBu* and *fCeBuBufr* (butyrate production from degradation of cellulose fraction of forage). For *de novo* production of propionate those that were most changed (35 to 48%) were *fCePrPrcn*, *fHcPrPrcn*, *fScPrPrcn* (propionate production from degradation of soluble carbohydrate fraction of concentrate), *fCePrPrfr* (propionate production from degradation of cellulose fraction of forage). The VFA stoichiometry coefficients of acetate that were most changed due to change in propionate and butyrate parameters in BASE model were for acetate production from soluble carbohydrate fraction of concentrate (49%). Since none of the parameters rested on the bound of +/- 75% of the original and the standard deviation of parameters were relatively small, the derived parameters seem to be well defined by the data.

The results of global sensitivity analysis of the parameters are presented in Table 4-3. The global sensitivity coefficient of acetate production was highest (0.835) for *KabsAc*, that of propionate was higher for *fStPrPrcn* (parameter for propionate production from starch fraction of concentrate) followed by *fAaFvPr* (parameter of propionate production from amino acid) in the VFAInt model (0.403 and 0.353, respectively). Butyrate production had higher sensitivity coefficient for *KabsBu* (rate constant for butyrate absorption) and *KabsAc* (0.496 and 0.316, respectively). Similarly in the BASE model, the sensitivity coefficient for acetate production was higher for *fAaFvPr* and *KabsAc* (0.295 and 0.215, respectively). The coefficient for propionate production was also higher for *fAcFvPr* and *fStPrPrcn* (0.43 and 0.42, respectively). The difference in sensitivity coefficient between VFAInt and BASE for acetate and butyrate should be because of the equations for interconversion of VFA that are based on thermodynamics. The interconversion of acetate and butyrate is extensive in the rumen and can range from 20% of acetate carbon from butyrate and 64% of butyrate carbon from acetate (Bergman et al., 1965, Sutton et al., 2003, France and Dijkstra, 2005). The free energy of the reactions is largely controlled by ruminal environment viz. pH, VFA concentration, partial pressure of the gas, ATP and ADP concentration. Since ADP and ATP concentration, and partial pressure of hydrogen were held constant and the VFA concentration are most sensitive to absorption parameters (Hanigan et al., 2013), the increased sensitivity of acetate and butyrate production in VFAInt model to absorption parameters is expected. Moreover, butyrate production in VFAInt model is also sensitive to *KabsAc* owing to the fact that carbon from acetate contributes significantly to net butyrate production as discussed above.

In general after the estimate, *de novo* propionate production decreased from all fraction of the diet in the VFAInt model but was decreased more for parameters which were not much sensitive to propionate production. The most sensitive parameters *fStPrPrcn* and *fAaFvPr* had modest reduction in final parameters (17 and 18% respectively) which resulted in slight reduction in predicted *de novo* production in VFAInt model but the mean predicted net production was increased. This was because of net gain of carbon by propionate, after the exchange with acetate and butyrate whose *de novo* and net production both were increased. The net gain however was minimal as expected because the conversion of propionate to acetate or butyrate has higher energy cost associated with it compared acetate and butyrate exchange (Ungerfeld and Kohn, 2006)

Though the inclusion of interconversions equations did not improve prediction in this study, over the years several studies have shown that the interconvensions are significant and can be variable across diets (Leng and Leonard, 1965a, Seal and Parker, 1994, Kristensen, 2001, Sutton et al., 2003). So, representing interconversion between VFA in a mechanistic cow model is imperative and thermodynamic influences have been shown as key driving forces for these interconversions (Kohn and Boston, 2000, Ungerfeld and Kohn, 2006, Ghimire et al., 2014). Moreover, our recent study in continuous culture has revealed that pH independently reduces the methane emission particularly by diverting reduced hydrogen more towards propionate production. In the same study we also found that the concentration of propionate influences acetate and propionate ratio in continuous culture. These effects are not represented in the BASE model and the later effect has been shown to be the result more propionate converting to acetate at higher propionate concentration (Seal and Parker, 1994). This indicates that our approach of representing interconversions should in theory capture the later shift in VFA production. Therefore, the large errors of prediction in this study even in VFAInt model could be because several other factors such as inaccurately defined rate constant due to assumed constants in the thermodynamic equations used in this study, poor prediction of passage rate as discussed above, inadequate representation of effect of pH on VFA stoichiometric coefficient, or errors associated with the reported production data in the dataset used for this study. The reactant and product concentration that are assumed constant in the thermodynamic equations used in this study for example hydrogen concentration, could vary depending upon the nature of the diet (Hegarty and Gerdes, 1999, Janssen, 2010). However, the data is scant in the literature to represent this variation. Similarly, the effect of pH on VFA stoichiometry coefficient in the Molly cow model is a discontinuous one (Baldwin, 1995). A continuous equation derived from wide range of pH on various diet types could better explain the effect of pH on the stoichiometry coefficient. The dataset used for this study was mostly from lactating cows which typically are not fed wide ranges in dietary concentrate proportions which limited our ability to derive such equation.

The large errors of predictions of VFA production can also be related to the heterogeneity of the data associated with the studies reporting VFA production rate. The effect of sampling locations on VFA production estimates in this dataset has been discussed previously (Ghimire et al., 2014). However, variation in reported VFA production rate could also have contributed to the errors. For example when the VFA production rate per unit of DM intake of all the treatment means in the VFA production rate data set were analyzed, 9 out of 23 treatment means had average production rate of 1.08±0.30 mol/d, 0.56±0.25 mol/d, and 0.30±0.18 mol/d of acetate, propionate and butyrate, respectively compared with 3.59±0.94 mol/d, 1.54±0.78 mol/d, and 0.85±0.34 mol/d of acetate, propionate and butyrate, respectively for rest of the data. This reveals that the lower reported production group represent only 30 to 36% of the VFA per kg of DM produced in the higher reported production group. These seemingly inaccurate estimation in the dataset included that of Markantonatos et al. (2008) (n=2), Markantonatos et al. (2009) (n=4), Armentano and Young (1983) (n=1) and Seal and Parker (1994) (n=2). Except for the Seal and Parker (1994) study all treatment means from this group had 25 to 70 % concentrate diet, so the possibility that the diet type would have resulted in such variation seems unlikely. Omitting these experiments would have however resulted limited power of the dataset used for the parameter estimation in this study.

With the regulatory compliance and safety issues, the use of ^{14}C labeled carbon in VFA studies is not preferred in recent years and the cost of stable isotope and the analyses involved is significantly higher which has led to fewer VFA metabolism studies over recent years.

Nevertheless, with the stable isotope being cheaper than it used to be before and the availability of IRMS in universities laboratories services should encourage more data acquisition in this respect. However, before that appropriate labeling of isotope should also be determined as they could result errors in estimates. For example, Markantonatos et al. (2008, 2009) used first position labeled isotopes for all the VFA in their studies and Armentano and Young (1983) used first position propionate which are labile and might get lost in other pools such as CO_2 and microbial carbon (Kristensen, 2001, France and Dijkstra, 2005). So, suitable isotopic positioning should be used while estimating VFA production with the isotope dilution technique. In this regard using culture experiments as a guide for animal experiments could be advantageous. It is easier to capture enrichment in all the pools (e.g., gas and microbes) in culture experiments and it requires relatively less amount of isotopes compared to animal experiments. Additionally, one of the limitations researchers face in using isotope dilution technique is that current models require the isotope to be in a steady state condition (France et al., 1987, France et al., 1991) which adds to the cost and length of the experiments. One such model was recently purposed by Nolan et al. (2014) using data from experiment conducted by Leng and Leonard (1965a) who used ^{14}C isotope for VFA production and interconversion estimates. In the paper, the authors also reported that the assumed steady state condition of tracer:tracee pool was not achieved in the original experiment and the proposed model would eliminate this source of variation. The attainment of steady state is the basis of production rate estimation in several of the literature used in the dataset for this study, so if such was the case in any of these studies the reported production rate would have been inaccurate. Moreover with the models used for VFA production studies, a buffer period is generally required to avoid carry over effect of isotope of one VFA to the other so if any such effect does occur, it will compromise the accuracy of the reported production. For example, Markantonatos et al. (2009) bolus dosed acetate and propionate together in one day followed by butyrate bolus the next day. If the exchange flux between acetate and propionate were different, the results could be biased. Furthermore, any carryover of acetate isotope would over estimate flux of butyrate to acetate and under estimate production of butyrate.

Conclusions

Including VFA interconversion equations based on thermodynamics in the Molly cow model, did not improve the predictions of VFA production as demonstrated by significantly lower LLF of VFAInt model when compared with BASE model after final parameter estimate. There was some reduction in prediction errors of VFA concentration after re-deriving the parameters but it was also evident in the model without interconversion equations indicating that these improvements were also not due to the newly introduced equations. An equation for effect of pH on VFA absorption was introduced in the model which did not help in reducing the errors of VFA concentration and production estimates. Potential inaccuracy of reported VFA production rate in the literature, and the assumptions included in thermodynamic equations due to scant data may have contributed to the errors. This underscores the importance of experiments that should be conducted to generate data on VFA production and driving variables that confer thermodynamic influence.

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Table 4-1. Residual error analyses for predictions by the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) before (initial) and after rederivation of parameters listed in Table 4-3 with (VFAInt) and without (BASE) representation of thermodynamically driven VFA[1] interconversions.

						Net production (mol/d)			De novo production (mol/d)		
	Ruminal pH	VFA, mol/L	Ac, mM	Pr, mM	Bu, mM	Ac	Pr	Bu	Ac	Pr	Bu
N	198	196	193	193	193	23	23	21	14	14	12
Observed	6.14	106.9	65.1	25.3	12.6	22.8	10.7	6.1	27.3	12.5	5.6
Predicted											
Initial	6.05	125.6	77.1	33	15.8	26.4	13.8	6.4	-	-	-
VFAInt	6.12	104.3	66.5	25.5	12.5	28.3	14.5	5.6	37.2	13.0	7.2
BASE	6.12	104.4	66.4	25.6	12.6	29.4	14.2	6.7	-	-	-
pHVFAInt[2]	6.12	103.9	63.1	29.3	11.7	29.6	14.2	5.6	37.3	13.5	7.2
pHBASE[3]	6.14	100.1	59.7	27.8	12.7	30.4	14.3	6.8	-	-	-
RMSPE[4] (% of observed mean)											
Initial	4.5	28.3	27.6	45.5	39.6	69.1	58.2	49.8	-	-	-
VFAInt	4.3	21.7	20.9	32.4	26.6	77.3	60	51.2	85	49.4	56.5

BASE	4.3	22	22.1	31.3	26.1	75.2	62.7	54.7	-	-	-
pHVFAInt	4.3	20.3	19.7	34.7	26.9	74	62.3	50.2	81.1	53.1	55
pHBASE	4.3	22.0	22.4	33.3	25.6	75.6	63.1	54.9	-	-	-
Mean bias (% of MSPE)											
Initial	12.9	38.6	44.3	43.6	40.8	5.1	25.3	0.9	-	-	-
VFAInt	0.4	1.2	1.1	0.0	0.2	9.9	35	2.5	18.5	0.7	27.8
BASE	0.4	1.1	0.7	0.1	0.04	14.6	27.3	3.5	-	-	-
pHVFAInt	0.3	2.0	3.2	20.5	6.6	16.0	28.5	2.8	20.0	2.5	28.1
pHBASE	0.0	8.2	13.7	8.8	0.1	19.4	27.6	4.5	-	-	-
Slope bias (% of MSPE)											
Initial	2.4	11.9	5.5	12.9	20.4	42.1	2.3	55.8	-	-	-
VFAInt	4.7	14.9	11.9	13.7	13.9	48.7	0.7	55.7	42.3	1.5	39.1
BASE	3.6	16.8	20.7	7.8	10.6	42.9	2.6	59.3	-	-	-
pHVFAInt	7.6	4.3	2.0	3.1	9.9	40.2	2.6	54.0	37.3	5.1	39.0
pHBASE	8.0	11.0	10.4	8.3	7.5	39.5	2.8	58.7	-	-	-

[1] VFA=volatile fatty acids, Ac=acetate, Pr=propionate, Bu=butyrate. [2] After including effect of pH on VFA absorption in VFAInt model. [3] After including effect of pH on VFA absorption in BASE model. [4] Root mean squared prediction error.

Table 4-2 Derived parameters both with (VFAInt) and without (BASE) equations to represent volatile fatty acids (VFA) interconversion in the Molly cow model with modification (Hanigan et al., 2006, 2009, 2013)

			BASE		VFAInt	
Model		Previous	BASE		VFAInt	
parameter	Description	value	estimate	SE	Estimate	SE
VFA stoichiometry coefficient^1						
*f*ScPrPrfr	Coefficient for degradation of soluble carbohydrate in forage to propionate	0.4	0.444	0.022	0.234	0.025
*f*StPrPrfr	Coefficient for degradation of starch in forage to propionate	0.34	0.361	0.025	0.327	0.031
f HcPrPrfr	Coefficient for degradation of hemi cellulose in forage to propionate	0.4	0.394	0.023	0.295	0.025
*f*CePrPrfr	Coefficient for degradation of cellulose in forage to propionate	0.2	0.279	0.022	0.152	0.016
*f*ScPrPrcn	Coefficient for degradation of soluble carbohydrate in concentrate to propionate	0.46	0.298	0.017	0.346	0.033
*f*StPrPrcn	Coefficient for degradation of starch in concentrate to propionate	0.7	0.639	0.047	0.582	0.046

*f*HcPrPrcn	Coefficient for degradation of hemi cellulose in concentrate to propionate	0.58	0.299	0.018	0.395	0.029
*f*CePrPrcn	Coefficient for degradation of cellulose in concentrate to propionate	0.2	0.110	0.010	0.139	0.012
*f*ScBuBufr	Coefficient for degradation of soluble carbohydrate in forage to butyrate	0.11	0.117	0.017	0.172	0.015
*f*StBuBufr	Coefficient for degradation of starch in forage to butyrate	0.23	0.262	0.023	0.351	0.025
*f*HcBuBufr	Coefficient for degradation of hemi cellulose in forage to butyrate	0.23	0.319	0.023	0.368	0.026
*f*CeBuBufr	Coefficient for degradation of cellulose in forage to butyrate	0.24	0.396	0.012	0.328	0.030
*f*ScBuBucn	Coefficient for degradation of soluble carbohydrate in concentrate to butyrate	0.32	0.179	0.013	0.158	0.017
*f*StBuBucn	Coefficient for degradation of starch in concentrate to butyrate	0.25	0.210	0.018	0.136	0.018
*f*HcBuBucn	Coefficient for degradation of hemi cellulose in concentrate to butyrate	0.15	0.163	0.013	0.099	0.011
*f*CeBuBucn	Coefficient for degradation of cellulose in concentrate to butyrate	0.11	0.157	0.011	0.102	0.013
*f*AaFvPr	Coefficient for degradation of amino acid to propionate	0.6	0.708	0.042	0.491	0.041
*f*AaFvBu	Coefficient for degradation of amino acid to butyrate	0.25	0.225	0.020	0.198	0.022

Rate constant for absorption						
KabsAc	Rate constant for acetate absorption	5.90593	9.462	0.172	9.3	0.255
KabsPr	Rate constant for propionate absorption	8.78725	13.517	0.178	13.329	0.254
KabsBu	Rate constant for butyrate absorption	7.95776	12.728	0.204	10.766	0.268

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[1] Value are in moles of VFA produced per mole of glucose or amino acid fermented, coefficient of acetate was calculated from difference, parameters were bound to +/- 75% of the previous value.

Table 4-3 Global sensitivity analyses of net production of volatile fatty acids (VFA) production to stoichiometry and absorption parameters (as described in Table 4-3) both with (VFAInt) and without (BASE) equations to represent VFA interconversion in the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013)

	Predicted VFA production[1]					
Model	BASE			VFAInt		
parameters	Ac	Pr	Bu	Ac	Pr	Bu
VFA stoichiometry coefficient						
ScPrPrfr	0.010	0.019	0.000	0.001	0.007	0.000
StPrPrfr	0.022	0.041	0.000	0.006	0.048	0.001
HcPrPrfr	0.002	0.004	0.000	0.000	0.004	0.000
CePrPrfr	0.001	0.002	0.000	0.000	0.001	0.000
ScPrPrcn	0.012	0.023	0.000	0.005	0.043	0.001
StPrPrcn	0.183	0.343	0.000	0.049	0.403	0.010
HcPrPrcn	0.003	0.006	0.000	0.002	0.017	0.000
CePrPrcn	0.001	0.001	0.000	0.000	0.003	0.000
ScBuBufr	0.002	0.000	0.010	0.001	0.000	0.004
StBuBufr	0.038	0.000	0.163	0.018	0.002	0.054

HcBuBufr	0.003	0.000	0.014	0.002	0.000	0.005
CeBuBufr	0.003	0.000	0.012	0.002	0.000	0.005
ScBuBucn	0.011	0.000	0.048	0.003	0.000	0.009
StBuBucn	0.065	0.000	0.280	0.007	0.001	0.022
HcBuBucn	0.003	0.000	0.014	0.000	0.000	0.001
CeBuBucn	0.002	0.000	0.010	0.000	0.000	0.001
AaFvPr	0.295	0.553	0.000	0.042	0.353	0.009
AaFvBu	0.073	0.000	0.418	0.013	0.002	0.061
Rate constant for absorption						
KabsAc	0.215	0.006	0.024	0.835	0.015	0.316
KabsPr	0.044	0.001	0.005	0.011	0.061	0.001
KabsBu	0.008	0.000	0.001	0.001	0.038	0.496

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[1] Ac= Acetate, Pr= Propionate, Bu= Butyrate

Figure 4-1 Residual errors of acetate, propionate, and butyrate concentration versus predicted NDF and starch (fraction of DM) using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) after estimates of parameters listed in Table 4-3 with the representation of VFA interconversion

Figure 4-2 Residual errors of net acetate, propionate, and butyrate production versus predicted NDF and starch (fraction of DM) using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) after estimates of parameters listed in Table 4-3 with the representation of VFA interconversion

Figure 4-3 Residual errors of acetate, propionate, and butyrate concentration versus predicted pH using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) after estimates of parameters listed in Table 4-3 with the representation of VFA interconversion

Figure 4-4 Residual errors of net acetate, propionate, and butyrate production versus predicted pH using the Molly cow model with modifications (Hanigan et al., 2006, 2009, 2013) after estimates of parameters listed in Table 4-3 with the representation of VFA interconversion

Figure 4-1 Ghimire et al.

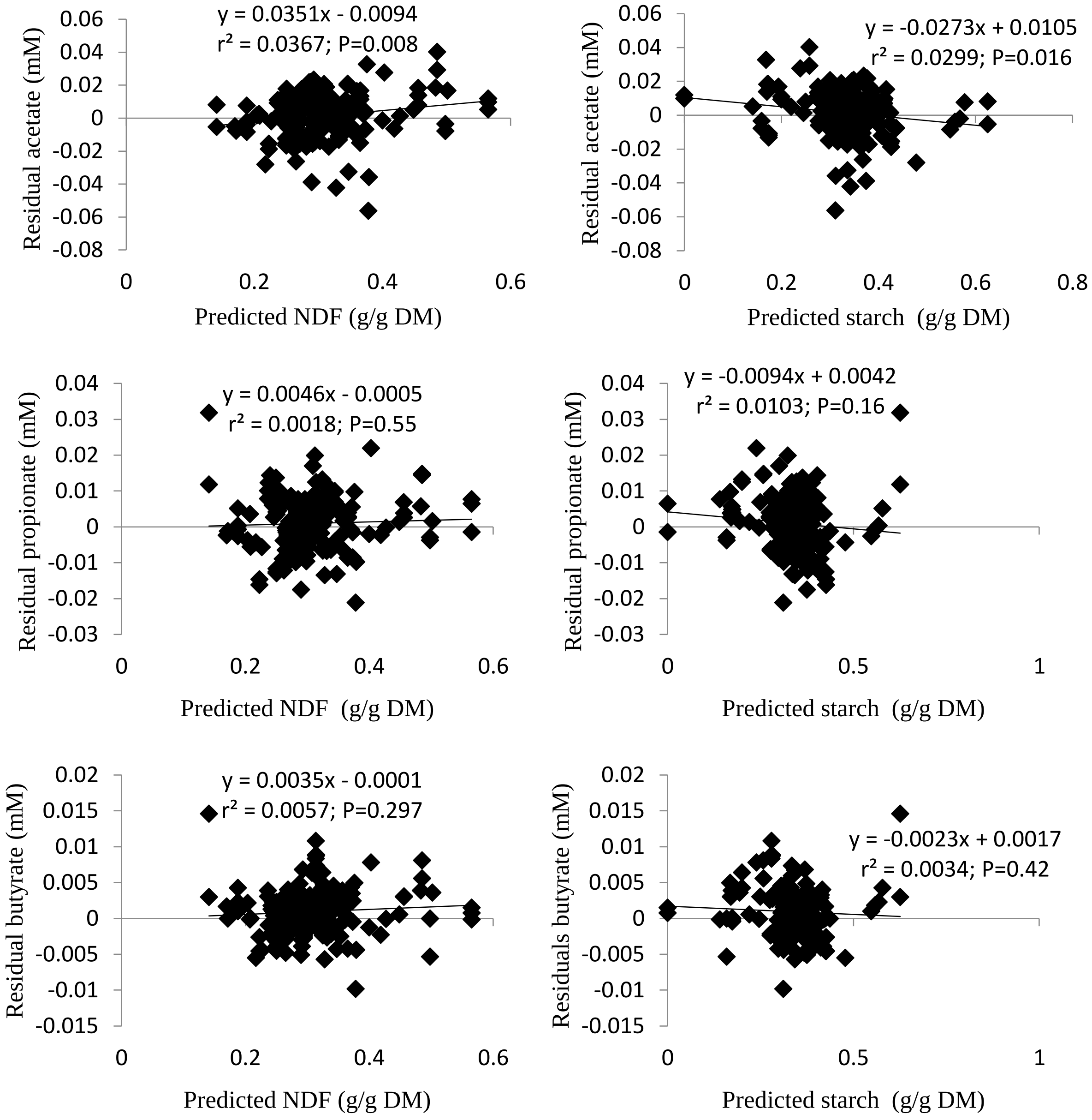


Figure 4-2 Ghimire et al.

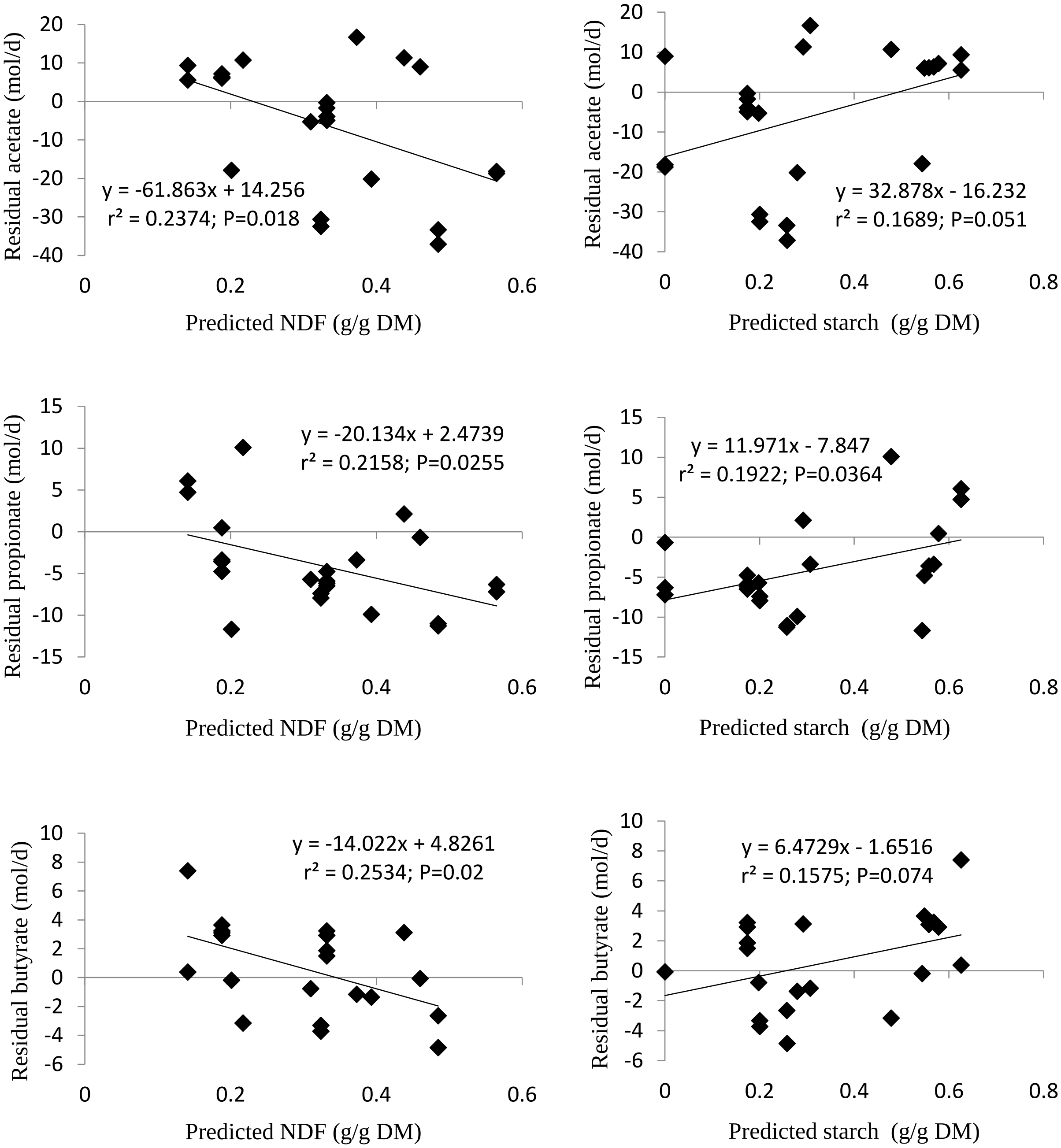
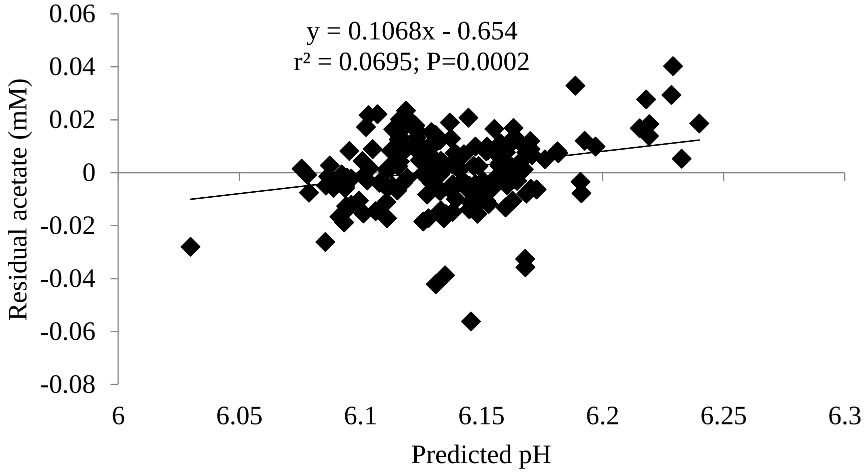
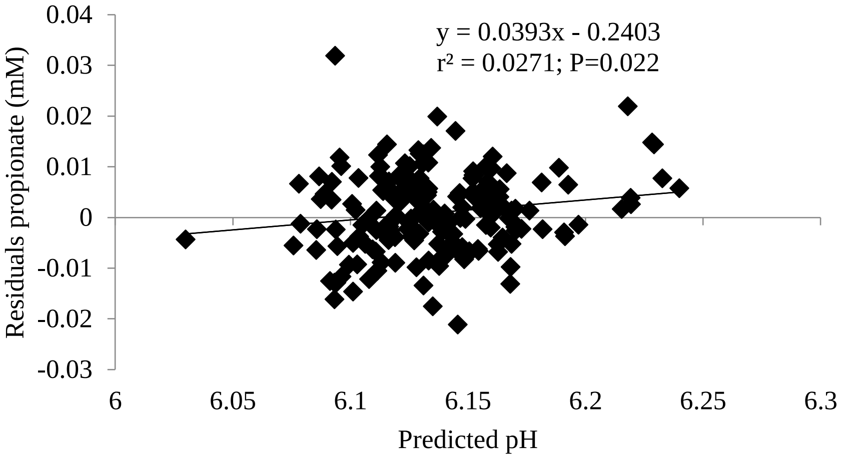


Figure 4-3 Ghimire et al.





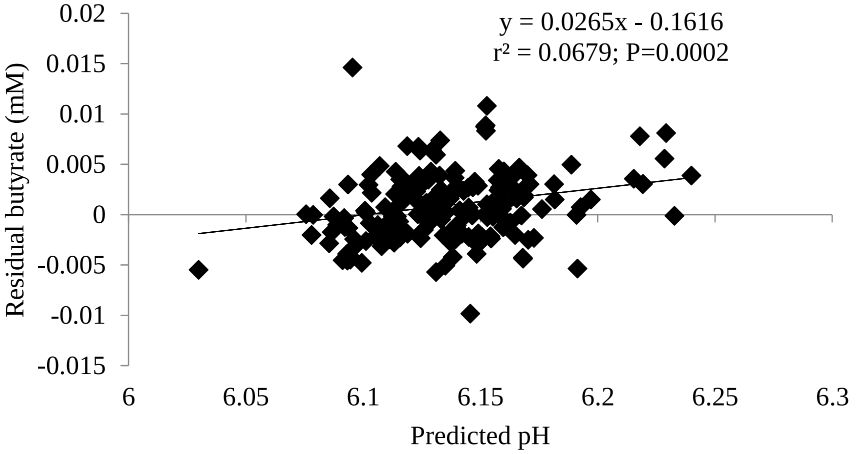
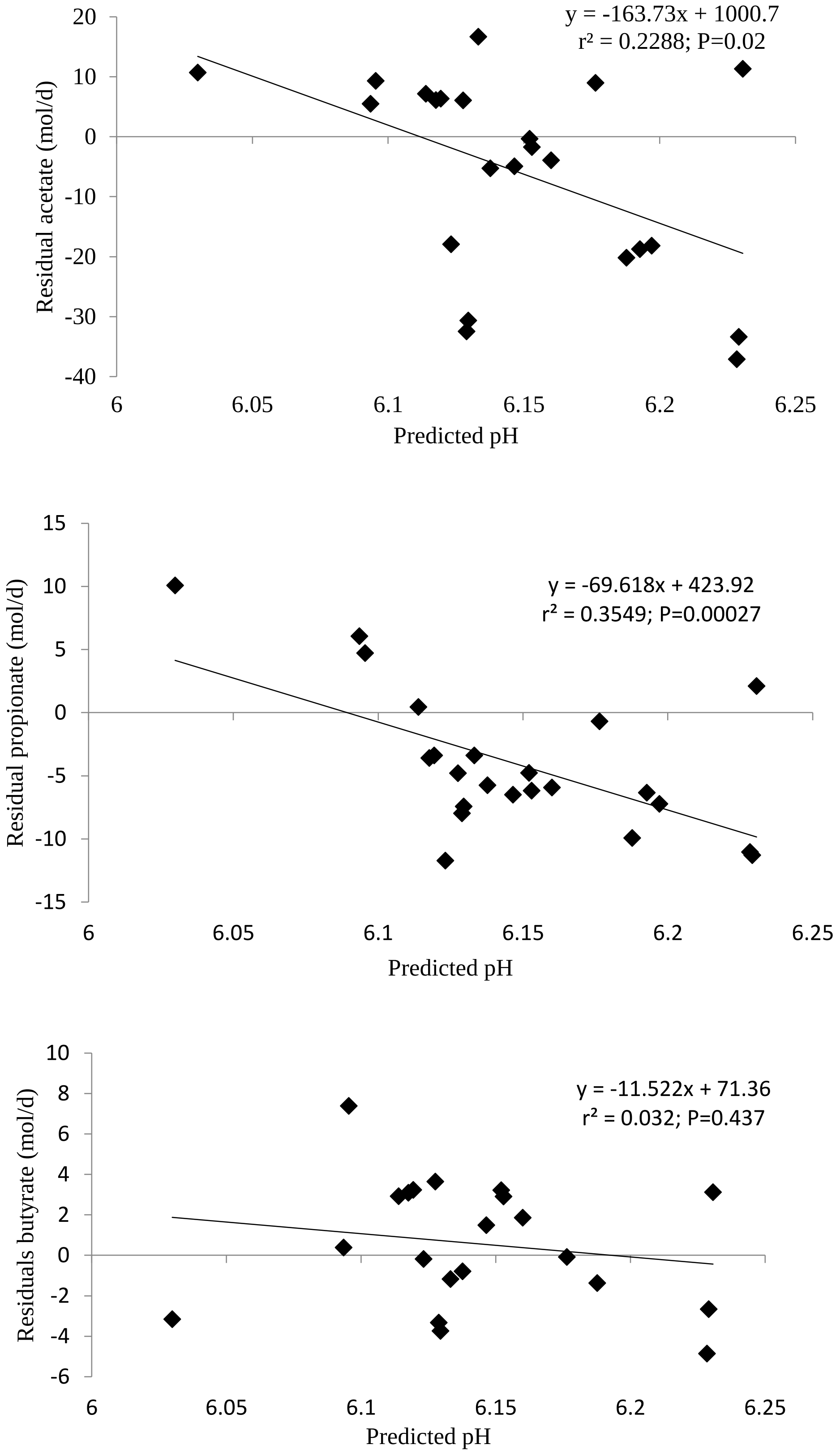


Figure 4-4 Ghimire et al.



CHAPTER 5 EFFECTS OF ACETATE, PROPIONATE, AND PH ON VOLATILE FATTY ACIDS AND METHANE PRODUCTION IN CONTINUOUS CULTURE

Abstract

Four continuous culture fermenters were used to determine the effects of acetate, propionate, and pH on VFA and methane production, and interconversions among VFA. The treatments were applied in the fermenters over four periods in a 4×4 Latin square design. The four treatments were: control, 20 mmol/d acetate infusion (INFAC), 7 mmol/d propionate infusion (INFPR), and low pH (LOWPH). For the LOWPH, buffer composition was adjusted to lower pH by 0.5 units compared to control (ranging from 6.62 to 6.97). The fermenters were fed 40 g of a pelleted 50:50 alfalfa to concentrate diet once daily. After 7 d of adjustment, 2-^{13}C sodium acetate at 0.29 g/d, $^{13}C_3$ sodium propionate at 0.29 g/d, and 1-^{13}C sodium butyrate at 0.024 g/d were separately infused continuously from 12 h before feeding for 36 h. Filtered liquid effluent (4 ml) was sampled at 0, 2, 4, 6, 8, 12, 16, and 22 h after feeding, and a pooled 24 h sample was collected for VFA analysis. Headspace methane and hydrogen gas were measure continuously. Production of propionate was higher in LOWPH ($P < 0.05$) compared to control and INFPR. The ratio of acetate to propionate production (AP ratio) in INFPR was higher than other treatments ($P < 0.05$). The AP ratio in LOWPH was lower than in control ($P < 0.05$). Methane production in LOWPH was lower ($P < 0.05$) than other treatments. Headspace hydrogen production was higher in INFAC, followed by INFPR and control, and LOWPH ($P < 0.05$). The VFA interconversions were not affected by treatments ($P > 0.05$). Around 29% of *de novo* acetate and 31% of *de novo* propionate production were estimated to be lost in the pools other than VFA. It was estimated that around 3% of *de novo* acetate was converted to propionate and 9% to butyrate. Exchange between propionate and butyrate was insignificant and below 1% of *de novo* production of either

VFA. Acetate and propionate concentrations and low pH did not affect VFA interconversions whereas low pH suppressed methane while increasing propionate production in continuous culture. The results also suggested that acetate can degrade to CO_2 and H_2 on ruminal environment in significant amounts and increased concentration of propionate affects the AP ratio by diverting the substrate towards propionate rather than oxidation of propionate to acetate.

Introduction

Volatile fatty acids (VFA) are important products of fermentation inside the rumen of ruminant animals. They are the chief source of energy to cows contributing up to 72% of the energy requirement (Bergman, 1990). Acetate, propionate, and butyrate are the major VFA and account 95% of the total VFA produced during fermentation. Stoichiometric patterns of these VFA influence release of reducing equivalent and aqueous concentrations of hydrogen which influence VFA stoichiometry via thermodynamics (Ungerfeld and Kohn, 2006).

Therefore, manipulation of these VFA can decrease methane emissions while maintaining animal productivity. Current VFA production predictions and subsequent methane emission predictions in a mechanistic model are based on the assumption of discrete sets of coefficients for each fraction of feed degraded to distinct VFA (Murphy et al., 1982, Dijkstra et al., 1992, Bannink et al., 2006). The effects of pH and VFA concentrations on VFA absorption has been described and predicted but their effects on *de novo* production (synonymously used as gross production hereafter) and VFA interconversions has not yet been quantified. Our recent study revealed that the current model does a poor job in predicting VFA production rate with 50-60% errors in prediction (Ghimire et al., 2014). Although in the second study in this dissertation indicated that addition of thermodynamic equations to represent interconversion among VFA did not improve the model, the errors could also have been due to variable reported VFA production data in the literature.

Although most of the models do not consider interconversions among VFA, isotopic dilution studies suggest that it does occur and is significant (Sutton et al., 2003). Since the conversion of pyruvate to acetate, propionate or butyrate share common intermediates and all of the reactions have negative free energy the flow of carbon among these VFA can be explained by thermodynamic control driven partially by ruminal pH and VFA concentration (Ungerfeld and Kohn, 2006). Therefore, increased concentration of one VFA could potentially affect H_2 supply for methanogenesis with more complicated process than described in previous methanogenesis models. Similarly, changes in ruminal pH can not only influence H_2 production and conversion to methane but also VFA interconversions. These facts indicate that the interconversions among VFA can be variable across diets and lack of representation of these effects might be the cause of inaccurate predictions of VFA production.

The hypothesis for this study was that the thermodynamic influence of varying pH and VFA concentration in the rumen alter VFA interconversions and result variable hydrogen production rates. The objective of the present study was to assess VFA production and interconversions among VFA under differing pH and VFA concentrations in a continuous culture.

Materials and Methods

Experimental Design and the Continuous Cultures

The study was conducted in four continuous culture fermenters similar to those described by Hoover et al. (1976) and modified by Hannah et al. (1986). The volume of the fermenters before the outflow ranged from 16, 56 ml to 18, 12 ml. The fermenters were connected to gas analyzers to measure CO_2 and H_2 production at 30 min intervals. The fermenters were fed 40 g of pelleted diet consisting of 50:50 alfalfa: concentrate once daily at 8:00 h. The ingredient composition of the diet is shown in Table 5-1. Treatments consisted of control, 20 mmol/d acetate infusion (INFAC), 7 mmol/d propionate infusion (INFPR), and low pH (LOWPH) applied over 4 periods in a 4×4 Latin square design. For LOWPH treatment the composition of normal buffer was altered to lower the pH in the fermenter by 0.5 units compared to control (Table 5-2). Ruminal inoculum for the experiment was obtained from 2 ruminally cannulated Jersey dairy cows fed lactating cow diet. The ruminal contents were filtered through double layered cheese cloth, placed in a thermos maintained at 39°C, and were taken to the lab where the contents from both cows were pooled and distributed to all the fermenters. The agitation rate of the fermenter was maintained at 50 rpm and the temperature was set to 39°C. The liquid and solid dilution rates were maintained 2.5% and 4.5%, respectively by adjusting the flow rate of buffer, treatment infusion, and filtrate removal rate. A multistage filter as described by Karnati et al. (2008) was used on the filtrate removal pump inlet to retain protozoa in the fermenters. The filtrate removal rate and buffer flow were monitored regularly during the day whereas infusion pumps were calibrated before the start of each period. A 7 d adjustment was provided before isotope infusion and sampling in each period of the study.

Isotope Infusion and Sampling

At d 7 of the experiment filtered liquid effluent (20 ml) from the fermenters was sampled at 12, 16, 22, 24, 26, 28, 30, and 32 h after feeding for measurement of aqueous hydrogen and methane concentrations. The samples were collected in crimp tight 60 ml vials containing 5 ml of 5 M H_3PO_4 flushed with nitrogen gas. On d 8 at 20:00 h isotope infusions were started with one of three ^{13}C-VFA (2-^{13}C sodium acetate at 0.29 g/d, $^{13}C_3$ sodium propionate at 0.29 g/d, and

1-^{13}C sodium butyrate at 0.024 g/d). The order of ^{13}C-VFA infusion was set by randomly assigning isotopes to the fermenters in the first period of the experiment and then for the later periods, the isotope to be infused was determined to make sure all treatments see all the sequences. The schedule of experiment, isotope infusions, and sampling was as shown in Table 5-2.

For aqueous hydrogen and methane, 20 ml LF samples were taken and immediately injected into a 60 ml vial, flushed with nitrogen gas, containing 5 ml of 5 M H_3PO_4, and capped with butyl rubber stopper with crimp seal. The LF samples (4 ml) from d 8 through d 14 of the experiment were used to determine the VFA concentration and enrichment. The samples were taken from the filtrate pump line with a 10 ml syringe, and transferred into a vial and immediately stored at -20°C until analysis. Additionally, the pH of the fermenters were measured from 2 ml LF sample taken at least one sampling point apart from d 9 through d 14, in such a way that all time points (0, 2, 4, 6, 8, 12, 16, 22 h after feeding) were covered. The pH samples were immediately dispensed into a 10 ml measuring cylinder and pH was measured using a portable pH meter. It was verified that the pH readings obtained from this method were matched to those obtained by directly dipping the probe of the pH meter into the fermenter.

The effluents collected during the sampling days were maintained on ice. Just before feeding on each day after the adaptation period, 7.5% of the effluent of the previous 24 h was collected for dry matter and nutrient content analysis. The samples from each treatment were later pooled separately by period and freeze dried. Samples of mixed pellets and individual pellets (alfalfa and concentrate pellets) were collected for each period and freeze dried, ground, and sent to Cumberland Valley Inc., MD for nutrient analysis. At the end of each ^{13}C-VFA infusion, 650 ml of mixed effluent samples were collected for microbial enrichment analysis and

DNA analysis. The samples for microbial enrichment were stored at -20°C until analysis whereas those for DNA analysis were stored at -80°C. About 4 ml of 24h pooled mixed effluent samples taken for microbial enrichment were later separated to analyzed VFA enrichment for measuring isotopic outflow in a day.

Sample Analysis

One of the outlets of the fermenter was connected to gas analyzers (Columbus Instruments, Columbus, OH) set to measure methane and hydrogen gas at 30 min intervals. The data thus obtained were analyzed for daily cumulative gas production and hourly production rate of headspace methane and hydrogen. For aqueous hydrogen and methane analysis, the headspace gas of the vials with LF was sampled with a gas tight syringe and individually injected into the analyzers.

Analysis for VFA concentration were done as described by Kristensen (2000). In short, ^{13}C isotope of acetate (1, 2-^{13}C sodium acetate), propionate (Sodium propionate-d_5), and butyrate ($^{13}C_4$ sodium butyrate) were used as internal standards and the VFA in the samples were esterified with chloroethyl chloroformate in a water, acetonitrile, and 2-chloroethanol solution. The derivetized samples were analyzed using a Thermo PolarisQ in tandem with a Focus GC. The GC was operated in split mode. A FactorFour VF-1701ms (30 m×.25 µm×.25 µm, Varian) column was used with a constant flow of Helium at 1.2 ml min^{-1}. A Selective Ion Monitoring (SIM) mass spectrum method in three segments running in positive electron ionization mode was used. Concentration data were used to calculate hourly production rates and 24h production. Isotopic enrichment analyses of VFA were done using a solid phase micro extraction (SPME) method with a Trace GC and Delta V-advantage isotope ratio mass spectrometer (Thermo Scientific, US). Sample of 450 µl was placed into a 20 ml glass vial and acidified with 50 µl of phosphoric acid. The vial was capped with butyl rubber stopper with crimp seal and placed for at least 12 minutes in a heating block set at 40°C. The SPME fiber was then exposed for one minute to the headspace gas produced in the vial through a syringe and then injected into the GC for 5 minutes. The temperature of GC inlet was set to 250°C with split less for 1 minute followed by split flow of 50 ml min^{-1}. A Zebron^{TM} capillary GC column (ZB-FFAP; 30 m×0.25 mm×0.25 μm) was used for separation with constant Helium flow of 1.5 ml min^{-1}. The oven was set at initial temperature of 32°C for 5 minutes followed by 7°C min^{-1} up to 200°C, and 15°C min^{-1} up to 240°C ramp.

To isolate the bacterial cells for enrichment analysis, the samples were thawed and blended in Waring blender at high speed for 1 minute to dislodge the bacteria in the solid particles. The blended fluid was filtered through four layers of cheese cloth and was centrifuged at 500 × g for 15 minutes. The bacteria were isolated from the supernatant by centrifuging at 23,000 × g for 15 minutes at 4°C. The precipitates thus obtained were purified by suspending with 0.9% saline solution and centrifuging again at 23,000 × g for 15 minutes at 4°C. The final precipitate was rinsed with distilled water and frozen which was later freeze dried and ground before analysis. Enrichment analysis of bacterial samples was carried out by an Elementar Isotope Cube Elemental Analyzer connected to an Isoprime 100 continuous flow isotope ratio mass spectrometer in the Isotope lab of the Geosciences Department at Virginia Tech.

Enrichment and Carbon Exchange Calculation

It was expected that after 12 h of isotope infusion, isotopic steady state would be achieved at the time of sample collection for VFA enrichment analysis (i.e. 0 h after feeding during sample collection day). However, results from 0, 12, and 22 h after feeding revealed that the concentration corrected enrichment levels were different and increasing over time.

Furthermore, the samples from time 0 h after feeding showed carryover of the isotopes when ^{13}C acetate or propionate preceded any other two VFA isotope in the infusion sequence indicating the enrichment in the fermenter did not reach background. Therefore the calculated carbon exchange and *de novo* synthesis per day were corrected for residual isotope for each day. This was calculated using the following equation

$$VFA^{*} = \frac{X_{ef}{}^{*}}{100} \times Prod \quad + \frac{X_{22}{}^{*}}{100} \times Con_{24} \times V - \frac{X_{0h}{}^{*}}{100} \times Con_{0} \times V$$

where VFA^* is the isotopic output from 24 h period of each VFA (mmol of ^{13}C VFA per day), X^* is the excess atom fraction (%) of total effluent ($X_{ef}{}^*$), 22 h LF sample ($X_{22}{}^*$), and *0h* LF sample ($X_0{}^*$) , *Prod* is the daily production of VFA for respective treatment (mmol/d), *Con* is the concentration of respective VFA (mM) at 24 h after feeding (*24*) and just before feeding (*0*), and *V* is the volume of fermenter (L) . The X^* was calculated as 100 × (AF_s –AF_b) where AF [^{13}C/ (^{12}C+^{13}C)] is the atomic fraction of *s* (sample) and *b* (background). The X^e at 22 h was assumed to be not different from X^* at 24 h and was taken at proxy X^*. The VFA^* for each of the VFA was then converted to the equivalent mmol of the ^{13}C VFA that was infused. For this conversion it was assumed that two moles of acetate are converted to one mol of butyrate, and one mol of butyrate converts to two moles of acetate. Similarly, it was assumed that one mol of acetate is required for its conversion to propionate and vice versa.

Data Analysis

The data were analyzed using Glimmix procedure of SAS® 9.3 (SAS Institute Inc., Cary, NC, USA). In the model, treatment and hour after feeding (when used) were included as fixed effects while fermenter and period were defined as random effects. Effect of treatment was declared significant when P <0.05.

Results

The pH for the LOWPH treatment was significantly lower ($P < 0.01$) than other treatments throughout the 24 h after feeding and the treatment was effective in reducing the pH by 0.5 units (Figure 5-1). The 24 h pH of LOWPH ranged from 6.06 at 12 h to 6.38 at 0 h. The INFPR and INFAC did not affect the pH ($P > 0.05$) in the fermenters. In all the treatments the pH began to decline immediately after feeding until 10 to 12 h after which there was a steady increase.

The results of nutrient digestibility are presented in Table 5-4 Effects of acetate, propionate and pH on nutrient digestibility in continuous culture. The digestibility of nutrients were not affected by treatments ($P > 0.05$). The mean digestibility of all treatments were 41, 48, and 81% for NDF, ADF, and starch, respectively.

The effects of treatments on VFA production are shown in Table 5-5. Acetate and butyrate production were not affected by treatments. Production of propionate was higher in LOWPH ($P < 0.05$) compared to control and INFPR. The hourly production of propionate was not different between treatments ($P > 0.05$) except at 2 h when it was higher in LOWPH compared to INFPR ($P < 0.05$). The ratio of acetate to propionate production (AP ratio) in INFPR was higher than other treatments ($P < 0.05$). The AP ratio of LOWPH was lower than control ($P < 0.05$).

Headspace methane production was lower ($P < 0.05$) in LOWPH compared to other treatments (Table 5-6). Methane production rate in LOWPH was lower ($P < 0.05$) than other treatments from 2 to 9 h, and was lower ($P < 0.05$) compared to INFAC and INFPR at 10 h. Methane production rate began to increase immediately after feeding and peaked at around 6 h followed by a gradual decline until 12 h after which the decline rate was slower (Figure 5-2).

Although the magnitude of production was lower, the pattern of methane production in the LOWPH followed that of other treatments.

Headspace hydrogen emission rates were higher in INFAC, followed by INFPR and control, and LOWPH (P <0.05). The emission rate of INFAC was higher (P <0.05) than others from 2 to 7 h. The hydrogen emission rate for all treatments increased rapidly after feeding and peaked at around 3 h after which it began to decline (Figure 5-3). After12 h the hydrogen emission rate for all treatments was negligible.

Aqueous methane concentrations were higher (P <0.05) in LOWPH compared to control and INFAC, whereas in INFPR it was higher than control (P <0.05). The differences were significant (P <0.05) at 16 h. The effect of treatment on aqueous hydrogen concentration was not significant (P >0.05).

The results of the VFA enrichments analysis revealed that the treatments did not affect interconversions among VFA (P >0.05). Conversion of acetate to butyrate and acetate to propionate was around 9% and 3 % of *de novo* acetate production, respectively whereas 25 to 30% of the *de novo* acetate was lost in pools other than propionate and butyrate (Table 5-7). There was minimal conversion of propionate to butyrate and was below 1% of *de novo* propionate production while conversion of propionate to acetate was 3% of *de novo* propionate production. The amount of infused $^{13}C_3$ propionate not recovered in acetate, propionate and butyrate was 30 to 33%. The recovery of butyrate exceeded the infused amount by 4 to 27 %. The estimated flux of butyrate to acetate was 34 to 45 % of *de novo* butyrate production. The conversion of butyrate to propionate was undetectable but indirect estimation from other fluxes revealed that 0.2-0.3 % of *de novo* butyrate production was converted to propionate.

Discussion

The diurnal pattern of pH in the fermenters matches that of *in vivo* studies with animals fed one meal per day such as those reported by Hünerberg et al. (2015). Though the entry rate of total VFA increased, we did not observe reduced pH in INFPR and INFAC in this study. Adding to the complexity is the fact that there is no neutralizing effect of bicarbonate dependent VFA absorption (Gabel et al., 2002) in the fermenters as is the case in rumen. Even though the published studies generally show reduced pH with increase in VFA concentration in the rumen, the relationship between VFA and pH seems to be weak (Dijkstra et al., 2012). However, it is important to note that VFA infusion in this study was continuous in contrast to VFA production due to highly fermentable diet which increases rapidly after feeding and then the production rate slows down. Additionally, the relationship derived in animal studies may have confounding effects since highly fermentable fraction of the feed can decrease the salivary secretion in the cow (Allen, 1997), whereas buffer flow in the fermenters were kept constant in this study. So not surprisingly, when acetate and propionate were separately infused in the rumen of lactating cows there was no effect on ruminal pH (Anil et al., 1993).

The results of the nutrient digestibility of LOWPH treatment are contrary to some other literature which reported depressed ruminal digestibly at low pH. For example Plaizier et al. (2001) reported around 20% decrease in 24 h NDF degradation in cows induced with sub-acute ruminal acidosis (SARA). However, Krajcarski-Hunt et al. (2002) observed depressed *in situ* NDF digestibility only in corn silage and not in grass hay and legume hay when incubated in SARA induced lactating cows. In the current study though the average 24 h pH in LOWPH was 6.24 which could have not been a critical pH to suppress pH sensitive fibrolytic bacterial and affect NDF digestibility. In a similar continuous culture study Calsamiglia et al. (2007) observed that low pH depressed NDF digestibility within the range of pH of 4.9 to 7 but from the equation they derived for 60:40 forage to concentrate diets predicts that NDF digestibility at pH 6.2 and 6.7 would not have been different (32 and 34%, respectively). Similarly, in Plaizier et al. (2001) study the average pH in SARA induced treatment was 5.87 which is 0.37 unit lower than this study. Grant and Weidner (1992) also suggested that critical pH for lag in NDF digestion is within a range of 6.2 to 5.8 depending upon the type of forages in the diet. The correlation between low pH and nutrient digestibility in animal studies can also be due to other factors such as passage rate. For example, highly fermentable diet can decrease pH but also increases the passage rate (Dijkstra et al., 2012) and thus passage rate could be the more influencing factor for NDF digestibility.

Propionate production in the LOWPH treatment was more than in control as expected. Increase in the molar proportion of propionate due to reduced pH have been reported (Hu et al., 2004). Calsamiglia et al. (2008) also found that reduced pH increased the production of propionate independent of the diet. The fermentation of starch is reported to be enhanced at lower pH with consequent increase in molar proportion of propionate (Marounek et al., 1985). In this study, however, the starch digestion in LOWPH was only numerically higher than control (82% vs 78%). It is suggested that at lower pH substrate degradation to propionate is thermodynamically more favorable than at higher pH (Ungerfeld and Kohn, 2006, Bannink et al., 2008). Consequently, the AP ratio of the LOWPH was lower than control and INFPR. Furthermore, increased production of propionate could also be related to changes in microbial communities. When SARA was induced with both alfalfa pellet and high starch diets, animals which were induced SARA with alfalfa pellet diets had higher population of *Prevotella spp*. specially *P. albensis* which have been shown to produce succinate and propionate as major end products (Khafipour et al., 2009). This shows that *Prevotella spp.* increase at low pH and is not dependent on availability of starch in the rumen. Therefore, it is possible that higher propionate production in LOWPH treatment in this study was because of increased population of *Prevotella spp*.

The diurnal pattern of methane production is similar to those reported by van Zijderveld et al. (2010) in sheep fed once a day where methane production peaked at 6 h after feeding and declined afterwards in control treatment. This pattern of production can be explained by declining amount of substrate to ferment over the course of the day after initial feeding. Methane production on LOWPH treatment was significantly lower than rest of the treatments (P <0.05) and is in agreement with other studies such as those reported by Russell (1998). The LOWPH reduced methane production probably by limiting reducing equivalent of H_2 thus hydrogen for methanogens through increased propionate production. Continuous oxidation of reducing equivalent is required for H_2 production which can then be utilized by the methanogens for methane production (Hegarty and Gerdes, 1999). It has been demonstrated that although the methanogenic archaeal communities differed on the solid and liquid phase of the rumen, the communities did not differ with pasture and TMR based diets (de Menezes et al., 2011). Therefore, reduction in methane production due to suppression of archaeal communities in this study is not expected.

The objective of including pH as a treatment in this study was to access its independent effect on VFA interconversion and net production. Although, propionate production was increased with reduced methane production due to low pH in the fermenters, infusion of acid in animals may not be feasible strategy to achieve such desirable response. In animals, adding acids in the rumen can cause problems such as depressed intake and altered absorption of minerals. For example, infusion of hydrochloric acid (0.35 M at 500 ml/d) in the rumen decreased the absorption of P and K in sheep (Giduck et al., 1988). When the pH of the rumen was reduced from 6.8 to 6.2 with hydrochloric acid or hydrochloric acid +sulfuric acid in alfalfa fed non lactating steers, the cows abruptly dropped the feed intake for 2 to 8 d (Esdale and Satter, 1972). For the same level of reduction in pH with phosphoric acid, the authors reported no change in feed intake.

Aqueous hydrogen concentration, though not significant, was lowest in LOWPH which could be because of less available hydrogen in the solution. The headspace and aqueous concentration of hydrogen do not follow the same pattern, which is expected. Because of the nonpolar nature of hydrogen gas, it is poorly soluble in the water and therefore the headspace and aqueous hydrogen does not normally equilibrate except in ruminal batch culture where the gas and liquid are in closed environment for a long time (Hegarty and Gerdes, 1999). The hourly production rate pattern of headspace hydrogen followed that of methane production (Figure 5-2 and Figure 5-3) but the differences among treatment for 24 h hydrogen emission and 24 h methane production were not similar. Nelson et al. (1960) also did not observe any changes in carbon dioxide to methane ratio when they rumen headspace of cows were sparged with hydrogen which indicates no change in methane production.

The increased AP ratio in INFPR in this study agrees with the results obtained by Seal and Parker (1994) in steers infused with 1 mol/d of propionate. They observed increased acetate production and attributed the increased ratio to oxidation of propionate to acetate. In this study, however acetate net production did not increase in INFPR treatment and enrichment analysis revealed that propionate to acetate conversion was also not affected. Thus, the increased AP ratio in INFPR should be due to substrate diversion to acetate in expense of propionate rather than due to propionate oxidation to acetate. One of the reasons for differences in results could be that the animals in Seal and Parker (1994) study were fed all grass pellet diets, an environment favorable for more acetate production. With high forage diet propionate production through succinate pathway is more than with concentrate diet (Baldwin et al., 1963). Since most of the degradation of propionate also occurs through succinate pathway (Schink, 1985), it is possible that with forage based diet more propionate degrades to acetate than with mixed diet. Sutton et al. (2003) also observed lesser carbon transfer from propionate (as percentage of total *de novo* propionate production) to acetate in low roughages diet compared to normal diet.

The INFAC did not affect the VFA production or acetate to propionate ratio when compared to control ($P > 0.05$). Significantly higher hydrogen emission and numerically higher methane production in the INFAC indicates that some of the added acetate were degraded to CO_2 and H_2, and also to methane. The breakdown of acetate to methane and CO_2 in anaerobic culture was reported by McInerney and Bryant (1981). The enrichment data also support these results where the recovery of infused 2-^{13}C acetate in all major VFA was 70 to 76% for all treatments indicating that some of it might have been lost in the gaseous pool. Kristensen (2001) estimated that about 28% of the acetate produced in the rumen exits the rumen in metabolites other than acetate. In the present study however, the amount of *de novo* acetate converted to other metabolites (including those not recovered) was estimated to be around 41 % and for propionate it was 35%. The difference in recovery of acetate could be because in animal study some of the labeled CO_2 from oxidation of acetate in peripheral tissues can be circulated back into the rumen which could increase the recovery of the isotope (Leng and Leonard, 1965a).

Even though it has been suggested that the process of VFA degradation in the rumen is normally too slow to have any significance (Fonty et al., 2007, Loughnan et al., 2014), it is proposed not be true (Kohn et al., 2015). For example, as suggested by the later author, at equilibrium the ΔG for acetate synthesis and degradation is near 0 and therefore the reaction is controlled by thermodynamics and changes in concentration of acetate could switch the reaction one way or the other. Having said that, the possibility of isotopic discrimination due to greater difference in molecular weight of uniformly labeled vs unlabeled propionate cannot be ruled out. Increased degradation of heavier molecular mass isotope has been observed (Leng and Leonard, 1965a). Klevenhusen et al. (2009) reported that ^{13}C can result in increased enrichment of CO_2 in the rumen simulation technique (Rusitec) system. Therefore low recovery of ^{13}C VFA isotope, particularly for uniformly labeled propionate in this study could be, to some extent, due to the preference of microbes for degrading heavier molecules.

In isotope dilution studies, position of carbon labeling of can affect the results and thus the interpretations. For example, Martin et al. (2001) used 1-^{13}C propionate as isotope dilution technique to estimate production. However, the first carboxyl-C is labile and when propionate converts to acetate, the labeled carbon can be lost resulting under estimation of propionate to acetate flux. Markantonatos et al. (2008, 2009) also used first position labeled isotopes for all the VFA in their studies and Armentano and Young (1983) used first position propionate which are labile and might get lost in other pools such as CO_2 and microbial carbon (Kristensen, 2001, France and Dijkstra, 2005). In this study it was assumed that one mol of propionate converts to one mol of acetate, thus even the carboxyl group of the $^{13}C_3$ propionate is lost, the other two carbons appearing in acetate pool is set equivalent to flux of one mol of propionate to acetate. In fact if all the pools are captured, uniformly labeled VFA can provide better assessment regarding carbon dynamics of VFA.   However, any isotope discrimination by microbes could affect uniformly labeled more, in proportion to the number of carbon labeled.

In this study about 9% of gross acetate production was converted to butyrate and 3% to propionate. This indicates that 39 to 52% of net butyrate production was derived from acetate. This is in agreement with data reported in sheep by Leng and Brett (1966) who estimated that 40 to 50% of butyrate was derived from acetate pool. Similarly, Weller (1967) also estimated that about 50% of butyrate was formed from the condensation of acetate molecules. Kristensen (2001) reported slightly larger flux from acetate to butyrate (16% of gross acetate production) in lactating cows but the flux from acetate to propionate and valerate was estimated to be 4 to 5% which is similar as found in this study. Lesser exchange of propionate carbon to other VFA were also reported in various other isotopic studies in animal where the exchange was usually less than 5% (Bergman et al., 1965, Esdale et al., 1968, Armentano and Young, 1983). Because less amount of 1-^{13}C butyrate was infused and there was much less flux from butyrate to propionate, the propionate enrichment from 1-^{13}C butyrate was undetectable, this could also be the reason why the amount of butyrate recovered was higher than what was infused. Indirect estimate from butyrate to acetate and acetate to propionate indicated that the flux from *de novo* butyrate production would only be 0.2 to 0.3%. However, Sutton et al. (2003) reported that 30 to 90% of *de novo* butyrate production converted to propionate which is in contrast to present study. This high level of exchange between butyrate and propionate cannot be easily explained given that the isotope positioning of butyrate was also similar to the present study. Since the VFA interconversions were not affected by VFA concentrations and pH in this study, the assumption of constant interconversions rate among VFA (as percentage of de novo), as assumed by Murphy et al. (1982), can be true. Having said that, differential rate of VFA absorption and passage rate in animal rumen might limit using VFA concentration as a direct estimator of the actual production.

Conclusions

In summary, VFA interconversions were not affected by pH, and acetate and propionate concentration in continuous culture as hypothesized. Significant interconversion between acetate and butyrate was observed whereas interconversion between butyrate and propionate was insignificant. Lowering pH by 0.5 units increased the production of propionate while decreasing methane production but did not affect nutrient digestibility. Infusion of acetate increased head space hydrogen production, indicating that acetate can be degraded to CO_2 and H_2 in ruminal environment.

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Table 5-1 Ingredient and nutrient composition of the experimental diet

Ingredient composition	% of DM
Alfalfa hay	50.0
Corn ground, rolled	18.4
Soybean flakes	20.2
Distillers dried grains with solubles	5.0
Soybean meal, 47%	4.5
Corn oil	0.90
Dicalcium phosphate 18.5%	0.39
Magnesium oxide 56%	0.10
Salt, trace mineralized	0.45
Nutrients	
ADF	25.9
NDF	31
Soluble CHO	4
Starch	14.5
NE_l (mcal/kg)	1.57

Table 5-2 Composition of the buffer used in the experiment*

Ingredients	Normal	Low pH
Sodium phosphate, dibasic, anhydrous (g)	35.25	32.25
Sodium bicarbonate (g)	100	100
Potassium chloride (g)	12	12
Potassium bicarbonate (g)	32	32
Urea (g)	8	8
5M Phosphoric acid (mL)	-	96
Distilled water (L)	20	20

*The buffers were reduced to pH of 6.75 (Normal) or 6.5 (Low pH) by constant bubbling CO_2 into the buffer solution.

Table 5-3. Schedule of the isotope infusion and sampling in the experiment

Day of experiment	Hour	Activities
Day 1	10:00 h	Rumen fluid inoculated into the fermenter
Day 3	8:00 h	Started infusing treatments
Day 7		Liquid filtrate (LF) samples taken for aqueous H and CH_4 at 12 and 16 h after feeding
Day 8	8:00h	The LF samples taken for aqueous H and CH_4 at -2 (equivalent to 22 h), 0, 2, 4, 6, and 8 h after feeding
	20:00 h	The LF samples taken and started 1st ^{13}C-VFA infusion
Day 9	8:00 h	LF sample taken just before feeding (0 h) and 2, 4, 6, 8, 12, 16, 22 h after feeding
Day 10	8:00 h	Infusion of 1st ^{13}C-VFA was stopped and replaced with water. Liquid and solid effluent were mixed and sampled
	10:00 h	The LF samples taken
	14:00 h	The LF samples taken
	20:00 h	Background sampling, water infusion stopped and 2nd ^{13}C-VFA infusion was started
Day 11 till day 14 8:00 h		Repeat procedure of day 9 and day 10 with 2nd and 3rd ^{13}C-VFA

Table 5-4 Effects of acetate, propionate and pH on nutrient digestibility in continuous culture

Digestibility (%)	Control	INFAC[1]	INFPR[2]	LOWPH[3]	SE	P value
NDF	38.5	42.1	40.7	41.5	3.9	0.51
ADF	44.5	48.2	48.2	52.4	4.5	0.34
Starch	78.2	80.6	81.2	82.4	1.8	0.28

[1] INFAC: Acetate infusion (20 mmol/d); [2] INFPR: Propionate infusion (7 mmol/d); [3] LOWPH: Buffer adjusted to lower pH by 0.5 units compared to control.

Table 5-5 Effects of acetate, propionate and low pH on VFA net productions in continuous culture*

VFA (mmol/d)	Control	INFAC[1]	INFPR[2]	LOWPH[3]	SE	P value
Acetate	91	87	92	89	4.7	0.67
Propionate	33.3^{b}	$34.4^{a,b}$	30.7^{b}	38.1^{a}	1.7	0.01
Butyrate	14.7	15.2	15.2	13.8	0.8	0.18
Acetate : Propionate	2.73^{b}	$2.55^{b,c}$	3.02^{a}	2.33^{c}	0.1	<0.01

*production was calculated after subtracting infused VFA from total entry rate. [1]INFAC: Acetate infusion (20 mmol/d); [2]INFPR: Propionate infusion (7 mmol/d); [3]LOWPH: Buffer adjusted to lower pH by 0.5 units compared to control. $^{a\text{-}d}$Means within a row with different superscripts differ ($P < 0.05$).

Table 5-6 Effect of acetate infusion, and propionate infusion and low pH on gaseous production and aqueous concentration of methane and hydrogen in continuous culture.

Gas production and concentration	Control	INFAC[1]	INFPR[2]	LOWPH[3]	SE	P value
Aqueous methane (uM)	120^{c}	130^{bc}	176^{ab}	187^{a}	18	0.02
Headspace methane (mmol/d)	41.47^{a}	47.43^{a}	42.23^{a}	24.77^{b}	9.54	<0.001
Aqueous hydrogen (uM)	2.25	1.74	2.01	1.47	0.31	0.35
Headspace hydrogen (umol/d)	$276.9^{b,c}$	604.7^{a}	$325^{a,b}$	80.4^{c}	127.7	<0.001

[1] INFAC: Acetate infusion (20 mmol/d); [2] INFPR: Propionate infusion (7 mmol/d); [3] LOWPH: Buffer adjusted to lower pH by 0.5 units compared to control.

Table 5-7 Gross production and interconversion[1] among VFA in continuous culture[2]

	Control	INFAC[3]	INFPR[4]	LOWPH[5]
Acetate				
Gross production (mmol/d)	158	138	159	160
Acetate to propionate				
mmol/d	4.1	3.6	4.2	6.8
% *de novo*	2.6	2.6	2.7	4.3
Acetate to butyrate				
mmol/d	13.7	11.9	14.6	14.4
% *de novo*	8.7	8.7	9.2	9.0
Isotope recovered	69	75	70	69
Propionate				
Gross production (mmol/d)	50.6	51.7	47.0	60.0
Propionate to acetate				
mmol/d	1.6	1.8	1.5	1.7
% *de novo*	3.2	3.4	3.2	2.8
Propionate to butyrate				
mmol/d	0.1	0.2	0.2	0.2
% *de novo*	0.3	0.5	0.5	0.3
Isotope recovered	69.2	70.4	68.9	66.7
Butyrate				
Gross production (mmol/d)	21.0	22.0	18.6	18.4
Butyrate to acetate				
mmol/d	7.0	9.3	8.5	6.2
% *de novo*	33.6	42.4	45.3	33.6
Butyrate to propionate[6]				
mmol/d	0.2	0.2	0.2	0.3
% *de novo*	0.9	1.1	1.2	1.4
Isotope recovered	104	112	127	109

[1] The interconversion flux among VFA was not affected by treatments (*P* >0.05).[2] Isotopes of VFA 2-^{13}C sodium acetate, ^{13}C3 sodium propionate, and 1-^{13}C sodium butyrate were separately infused for gross production and interconversion determination, the assumption for flux calculation was that two moles acetate condenses into butyrate, and one mol of butyrate splits into two moles of acetate. [3] INFAC: Acetate infusion (20 mmol/d); [4] INFPR: Propionate infusion (7 mmol/d); [5] LOWPH: Buffer adjusted to lower pH by 0.5 units compared to control.[6] Isotopic data on butyrate to propionate conversion were not detectable and were therefore derived after considering butyrate to acetate and acetate to propionate conversion.

Figure 5-1. The pH in different treatments (INFAC=20 mmol/d acetate infusion, INFPR= 7 mmol/d propionate infusion, LOWPH= pH reduced by 0.5 units compared to control) in continuous culture fermenters.

Figure 5-2. Head space methane production in different treatments (INFAC=20 mmol/d acetate infusion, INFPR= 7 mmol/d propionate infusion, LOWPH= pH reduced by 0.5 units compared to control) in continuous culture fermenters.

Figure 5-3. Head space hydrogen production in different treatments (INFAC=20 mmol/d acetate infusion, INFPR= 7 mmol/d propionate infusion, LOWPH= pH reduced by 0.5 units compared to control) in continuous culture fermenters.

Figure 5-1 Ghimire et al.

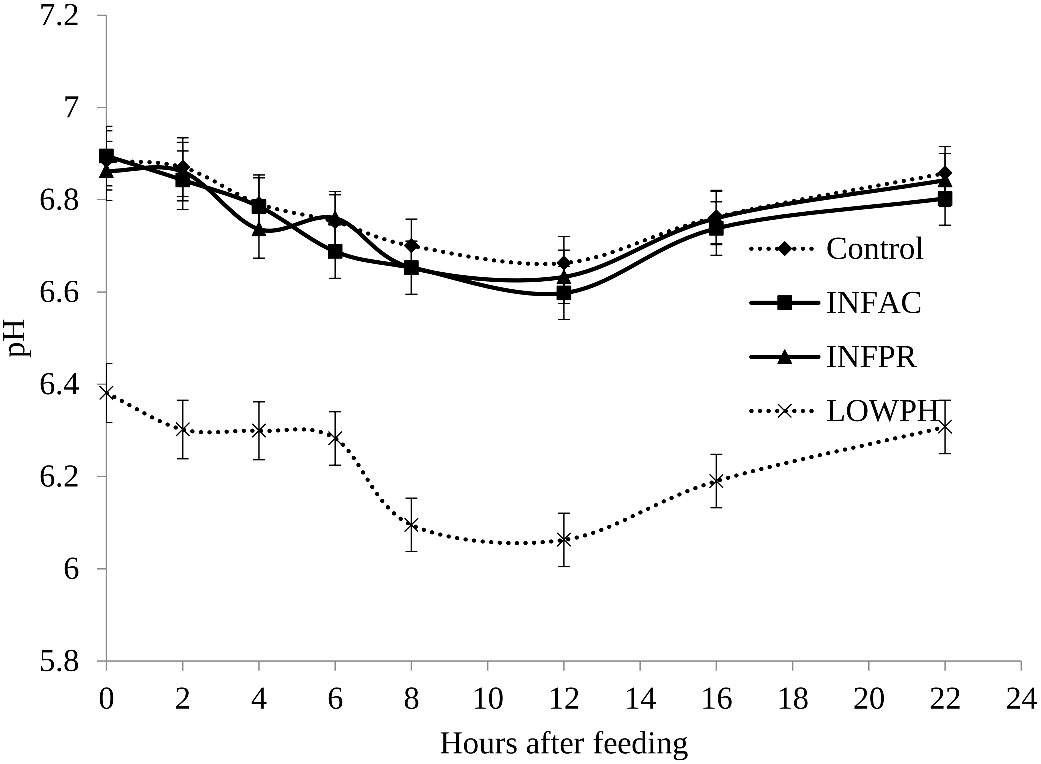


Figure 5-2 Ghimire et al.

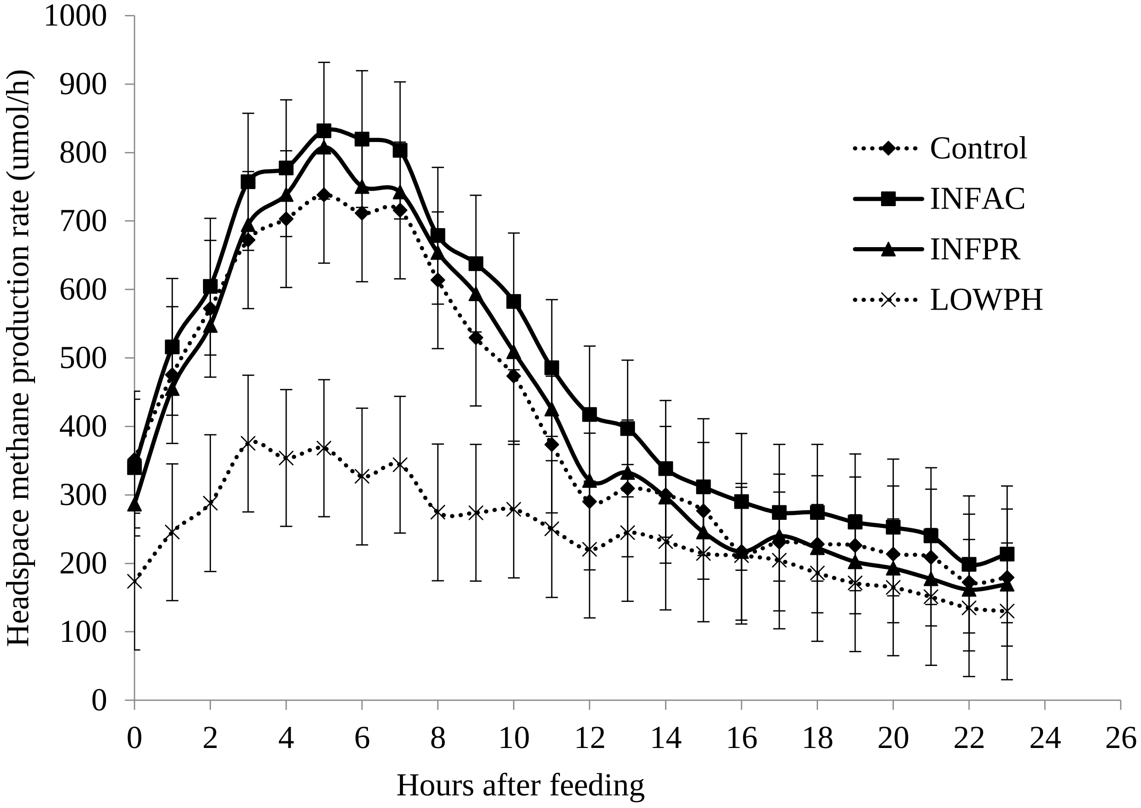
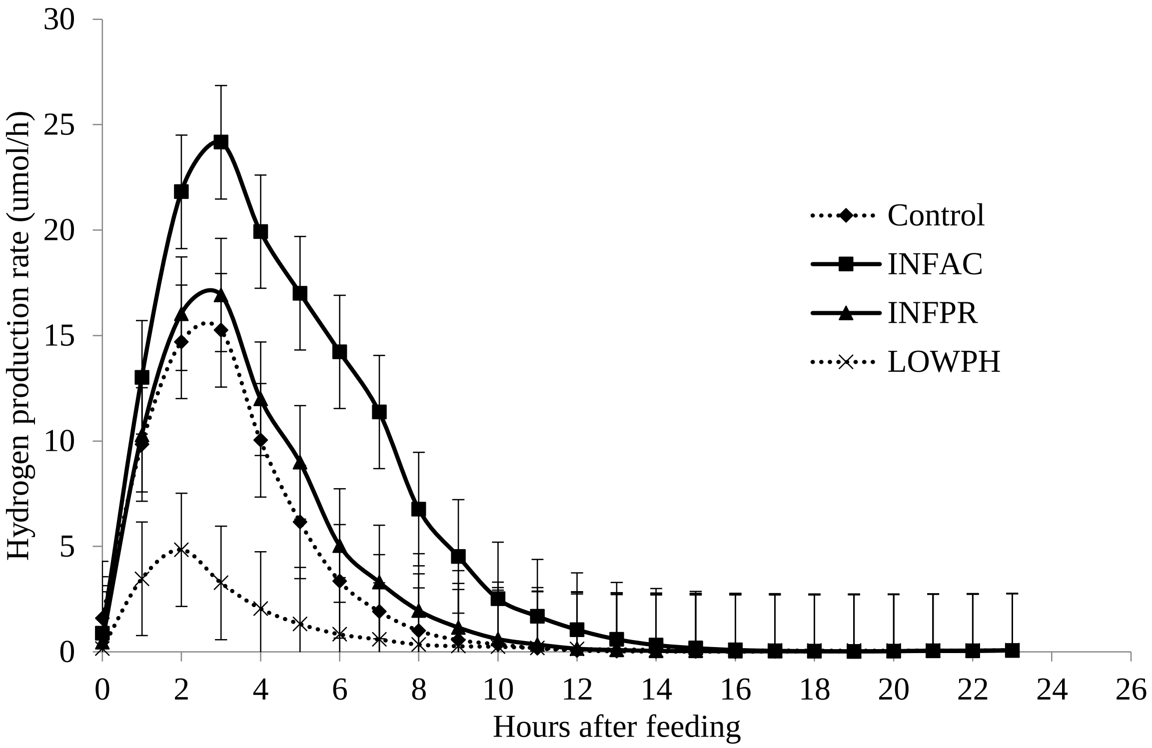


Figure 5-3 Ghimire et al.



CHAPTER 6 OVERALL CONCLUSIONS

The studies conducted for this dissertation were designed to test the hypothesis that interconversions among VFA are controlled by the thermodynamic influence of VFA concentration and pH, and representing it in a mechanistic model will improve the prediction of VFA production. Firstly, we evaluated a mechanistic cow model, Molly, in terms of its prediction of VFA production, and later represented VFA interconversions in the model to improve its predictions (**Chapter 2 and Chapter 3**).

The evaluation of the Molly cow model indicated that the model did not predict VFA production accurately with more than 50% errors in predictions. Preliminary assessment revealed that the interconversion may be partially controlled by thermodynamic effect of VFA concentration and pH. In the subsequent study, equations based on thermodynamics were introduced in the model to represent interconversions among VFA. Parameters of *de novo* VFA production and VFA absorption were re-estimated and evaluations were done in both original model and the modified model using 8 studies reporting pH, VFA concentration, and VFA production rates. Introducing interconversions among VFA did not improve the model predictions of VFA production with RMSPE of 77, 60 and 51% for acetate, propionate and butyrate, respectively. Including the effect of pH on VFA absorption reduced the mean bias of propionate production and slope bias of acetate production but not the overall RMSPE. It was concluded that the larger prediction errors for VFA production could be due to measurement variation, poor representation of the effects of pH on stoichiometric parameters, or incorrect rate constants for VFA interconversions.

Secondly, an experiment was conducted to assess VFA production and interconversions among VFA under differing pH and VFA concentrations in a continuous culture using stable isotopes of acetate, propionate and butyrate in a continuous culture.

Increased concentration of acetate and propionate did not affect interconversion fluxes among VFA. We observed significant interconversion between acetate and butyrate, however the interconversion between propionate and butyrate was insignificant. Lowering the pH by 0.5 units increased the production of propionate and reduced methane emissions, the effect on VFA interconversion was however not significant. Our results also revealed that the degradation of VFA in the continuous culture was significant with about 30% of *de novo* acetate and propionate production not accounted by the isotopic recovery. Infusion of acetate increased head space hydrogen production, indicating that acetate can be degraded to CO_2 and H_2 in ruminal environment. These results revealed that thermodynamics influence of VFA concentration and pH were not conferring variable interconversions among VFA as hypothesized.

APPENDIX

Headspace Gas in the Fermenters

Period	Fermenter	H_2 Rate[1]	H_2 accumulated[2]	CH4 rate	CH_4 accumulated[2]	Time	Hour after feeding	Sampling day
1	1	0.173	986.988	242.069	74274.63	8	0	8
1	1	0.248	987.118	253.263	74407.31	8	0	8
1	1	0.012	1473.93	64.998	83051.87	8	0	8
1	1	0.017	1473.939	172.083	83140.16	8	0	8
1	1	0.503	987.382	411.298	74622.9	9	1	8
1	1	5.224	990.181	641.173	74966.46	9	1	8
1	1	0	1473.939	127.083	83206.8	9	1	8
1	1	0.295	1474.093	396.127	83414.33	9	1	8
1	1	64.536	1024.726	681.073	75331.02	10	2	8
1	1	64.796	1059.391	795.206	75756.46	10	2	8
1	1	1.478	1474.885	613.622	83742.79	10	2	8
1	1	3.734	1476.882	536.614	84029.88	10	2	8
1	1	64.871	1094.133	814.003	76192.41	11	3	8
1	1	64.658	1128.761	719.051	76577.5	11	3	8
1	1	7.369	1480.825	589.224	84345.11	11	3	8
1	1	10.26	1486.319	685.488	84712.23	11	3	8
1	1	64.338	1163.218	587.794	76892.3	12	4	8
1	1	65.707	1197.641	581.527	77196.95	12	4	8
1	1	10.999	1492.216	750.682	85114.67	12	4	8
1	1	64.4	1232.184	681.046	77562.26	13	5	8
1	1	64.715	1266.825	771.797	77975.38	13	5	8
1	1	12.668	1498.993	857.325	85573.34	13	5	8
1	1	13.463	1506.203	887.935	86048.88	13	5	8
1	1	64.845	1301.517	817.751	78412.88	14	6	8
1	1	64.859	1336.216	823.852	78853.64	14	6	8
1	1	14.34	1513.895	940.373	86553.29	14	6	8
1	1	14.602	1521.719	978.099	87077.39	14	6	8
1	1	64.826	1370.898	807.441	79285.63	15	7	8
1	1	13.564	1528.979	983.501	87603.84	15	7	8
1	1	9.431	1534.033	806.76	88036.13	15	7	8
1	1	52.368	1398.929	702.94	79661.89	16	8	8
1	1	32.862	1416.529	520.728	79940.77	16	8	8
1	1	3.441	1535.876	340.492	88218.48	16	8	8
1	1	1.352	1536.6	174.532	88311.95	16	8	8
1	1	20.896	1427.708	403.921	80156.87	17	9	8
1	1	16.977	1436.805	367.809	80353.95	17	9	8
1	1	1.003	1537.137	167.69	88401.8	17	9	8

1	1	1.103	1537.728	204.039	88511.02	17	9	8
1	1	15.219	1444.951	382.355	80558.62	18	10	8
1	1	11.541	1451.125	345.604	80743.52	18	10	8
1	1	1.378	1538.466	272.515	88657.04	18	10	8
1	1	1.333	1539.18	290.198	88812.45	18	10	8
1	1	10.193	1456.578	356.52	80934.25	19	11	8
1	1	8.192	1460.972	337.718	81115.4	19	11	8
1	1	1.387	1539.923	334.501	88991.59	19	11	8
1	1	1.062	1540.491	296.278	89150.27	19	11	8
1	1	6.466	1464.362	331.4	81289.11	20	12	8
1	1	5.025	1466.997	297.365	81445.06	20	12	8
1	1	0.998	1541.026	329.768	89326.88	20	12	8
1	1	3.373	1468.803	237.688	81572.36	21	13	8
1	1	2.788	1470.264	239.304	81697.73	21	13	8
1	1	0.953	1541.526	398.065	89535.64	21	13	8
1	1	0.591	1541.843	363.231	89730.17	21	13	8
1	1	2.251	1471.444	238.119	81822.54	22	14	8
1	1	1.537	1472.25	215.425	81935.52	22	14	8
1	1	0.397	1542.051	343.611	89910.38	22	14	8
1	1	0.19	1542.151	278.93	90056.73	22	14	8
1	1	1.056	1472.805	198.887	82039.98	23	15	8
1	1	0.164	1542.237	301.493	90214.77	23	15	8
1	1	0.13	1542.305	315.002	90379.97	23	15	8
1	1	0.723	1473.184	178.852	82133.78	0	16	8
1	1	0.476	1473.434	167.654	82221.7	0	16	8
1	1	0.183	1473.528	132.468	82289.66	1	17	8
1	1	0.241	1473.652	68.877	82325.02	1	17	8
1	1	0.179	1473.743	148.641	82401.28	2	18	8
1	1	0.125	1473.808	64.555	82434.42	2	18	8
1	1	0.149	1473.884	175.168	82524.29	3	19	8
1	1	0.026	1473.898	156.48	82606.35	3	19	8
1	1	0.047	1473.921	52.973	82632.94	4	20	8
1	1	0.006	1473.925	159.907	82716.8	4	20	8
1	1	-0.001	1473.924	149.129	82793.3	5	21	8
1	1	0.003	1473.926	57.427	82822.77	5	21	8
1	1	-0.001	1473.925	165.097	82907.52	6	22	8
1	1	-0.001	1473.924	139.534	82980.7	6	22	8
1	1	0	1473.924	48.554	83005.07	7	23	8
1	1	0	1473.924	26.76	83018.5	7	23	8
1	1	0.056	1542.553	209.484	92530.72	8	0	9
1	1	0.057	1542.582	229.685	92648.56	9	1	9
1	1	0.031	1542.598	103.707	92702.89	9	1	9
1	1	0.01	1542.603	21.93	92714.14	10	2	9

1	1	0.033	1542.621	126.459	92780.46	10	2	9
1	1	0.33	1542.794	225.081	92898.5	11	3	9
1	1	3.122	1544.466	135.189	92970.9	11	3	9
1	1	3.576	1546.339	62.806	93003.8	12	4	9
1	1	36.014	1565.616	338.295	93184.88	12	4	9
1	1	65.056	1600.512	493.911	93449.81	13	5	9
1	1	66.194	1635.962	663.969	93805.41	13	5	9
1	1	66.125	1671.376	742.407	94203.01	14	6	9
1	1	65.996	1706.739	758.34	94609.35	14	6	9
1	1	65.789	1741.972	776.485	95025.2	15	7	9
1	1	54.594	1771.21	781.667	95443.83	15	7	9
1	1	46.106	1795.416	808.496	95868.29	16	8	9
1	1	38.269	1815.889	811.586	96302.48	16	8	9
1	1	32.491	1833.29	845.36	96755.22	17	9	9
1	1	21.825	1844.754	614.592	97078.05	18	10	9
1	1	11.284	1850.797	411.643	97298.51	18	10	9
1	1	12.023	1856.969	533.819	97572.53	19	11	9
1	1	10.924	1862.695	568.322	97870.43	19	11	9
1	1	10.43	1868.275	631.838	98208.46	20	12	9
1	1	9.114	1873.05	628.849	98537.91	20	12	9
1	1	7.437	1876.952	594.727	98849.97	21	13	9
1	1	6.137	1880.099	577.307	99146	21	13	9
1	1	4.877	1882.655	546.451	99432.43	22	14	9
1	1	3.603	1884.503	467.936	99672.38	22	14	9
1	1	2.739	1885.938	443.405	99904.8	23	15	9
1	1	1.87	1886.898	364.318	100091.7	23	15	9
1	1	0.089	1542.351	310.569	90542.84	0	16	9
1	1	0.062	1542.384	294.702	90697.4	0	16	9
1	1	0.024	1542.396	259.497	90833.42	1	17	9
1	1	0.01	1542.401	256.609	90968	1	17	9
1	1	0.006	1542.404	256.621	91102.44	2	18	9
1	1	0.006	1542.407	254.998	91233.27	2	18	9
1	1	0.002	1542.409	244.526	91361.51	3	19	9
1	1	0.003	1542.411	236.597	91485.59	3	19	9
1	1	0.009	1542.415	244.668	91611.12	4	20	9
1	1	0.009	1542.42	223.577	91728.37	4	20	9
1	1	0.016	1542.428	218.118	91840.27	5	21	9
1	1	0.026	1542.441	225.137	91958.41	5	21	9
1	1	0.042	1542.463	261.159	92092.4	6	22	9
1	1	0.039	1542.483	230.942	92213.52	6	22	9
1	1	0.03	1542.499	186.989	92311.58	7	23	9
1	1	0.048	1542.524	213.007	92420.86	7	23	9
1	1	0.002	1889.274	236.647	102396.2	8	0	10

1	1	0.009	1889.278	170.076	102485.4	8	0	10
1	1	0.001	1889.279	104.216	102538.9	9	1	10
1	1	0.152	1889.357	251.353	102667.9	9	1	10
1	1	6.88	1893.045	216.153	102783.8	10	2	10
1	1	44.202	1916.693	446.306	103022.6	10	2	10
1	1	65.793	1951.198	683.028	103380.8	11	3	10
1	1	65.025	1986.022	938.087	103883.2	11	3	10
1	1	66.724	2020.979	1096.259	104457.5	12	4	10
1	1	65.416	2056.013	1132.236	105063.9	12	4	10
1	1	66.719	2091.003	1123.495	105653.1	13	5	10
1	1	53.652	2119.737	1144.722	106266.2	13	5	10
1	1	45.731	2144.253	1167.027	106891.8	14	6	10
1	1	40.131	2165.724	1186.976	107526.9	14	6	10
1	1	34.84	2183.986	1164.068	108137	15	7	10
1	1	27.905	2198.915	1058.831	108703.5	16	8	10
1	1	22.175	2210.532	935.518	109193.6	16	8	10
1	1	17.086	2219.683	840.059	109643.5	17	9	10
1	1	14.38	2227.221	796.205	110060.9	17	9	10
1	1	11.179	2233.077	729.5	110443	18	10	10
1	1	8.917	2237.751	682.118	110800.6	18	10	10
1	1	6.815	2241.327	631.563	111132	19	11	10
1	1	4.934	2243.914	569.229	111430.3	19	11	10
1	1	3.316	2245.653	487.873	111686.2	20	12	10
1	1	2.622	2247.027	505.199	111951	20	12	10
1	1	1.598	2247.865	422.226	112172.4	21	13	10
1	1	0.933	2248.343	333.205	112343.3	21	13	10
1	1	0.763	2248.743	385.844	112545.6	22	14	10
1	1	0.493	2248.997	369.816	112735.4	22	14	10
1	1	0.288	2249.145	342.435	112911.2	23	15	10
1	1	0.165	2249.229	338.898	113085	23	15	10
1	1	1.193	1887.509	292.181	100241.5	0	16	10
1	1	1.107	1888.077	369.533	100431.1	0	16	10
1	1	0.83	1888.503	371.567	100621.8	1	17	10
1	1	0.55	1888.785	327.322	100789.6	1	17	10
1	1	0.375	1888.977	311.586	100949.5	2	18	10
1	1	0.288	1889.128	327.045	101121	2	18	10
1	1	0.118	1889.189	254.5	101251.7	3	19	10
1	1	0.074	1889.228	249.13	101382.2	3	19	10
1	1	0.058	1889.258	266.7	101522	4	20	10
1	1	0.01	1889.263	231.016	101643.2	4	20	10
1	1	0.014	1889.27	257.733	101778.4	5	21	10
1	1	0.004	1889.272	255.549	101912.4	6	22	10
1	1	0.001	1889.273	234.651	102035.5	6	22	10

1	1	-0.001	1889.272	237.713	102157.4	7	23	10
1	1	0.001	1889.273	223.506	102274.6	7	23	10
1	1	-0.004	2249.207	214.797	114910.3	8	0	11
1	1	-0.004	2249.204	200.048	115013	8	0	11
1	1	-0.003	2249.203	150.612	115090.3	9	1	11
1	1	0.199	2249.303	294.227	115238	9	1	11
1	1	6.682	2252.882	622.659	115571.4	10	2	11
1	1	33.573	2270.843	815.976	116008	10	2	11
1	1	65.694	2305.26	985.778	116524.4	11	3	11
1	1	66.619	2340.179	985.599	117041	11	3	11
1	1	59.556	2372.042	972.773	117561.5	12	4	11
1	1	51.983	2399.881	1188.514	118198	12	4	11
1	1	38.367	2419.992	1129.735	118790.2	13	5	11
1	1	26.588	2434.224	1032.802	119343	13	5	11
1	1	24.583	2447.123	1141.594	119942	14	6	11
1	1	22.17	2458.99	1187.195	120577.5	14	6	11
1	1	18.316	2468.601	1112.962	121161.5	15	7	11
1	1	15.017	2476.635	1080.003	121739.3	15	7	11
1	1	13.297	2483.609	1087.887	122309.8	16	8	11
1	1	9.799	2488.747	912.161	122788.2	17	9	11
1	1	6.763	2492.292	739.713	123175.9	17	9	11
1	1	4.663	2494.738	618.391	123500.3	18	10	11
1	1	4.459	2497.076	705.577	123870.3	18	10	11
1	1	3.6	2498.964	777.363	124278	19	11	11
1	1	2.493	2500.27	647.528	124617.2	19	11	11
1	1	1.52	2501.067	548.735	124904.8	20	12	11
1	1	0.984	2501.582	496.663	125165	20	12	11
1	1	0.636	2501.908	461.855	125402	21	13	11
1	1	0.331	2502.082	402.645	125612.9	21	13	11
1	1	0.151	2502.159	362.494	125798.9	22	14	11
1	1	0.069	2502.195	354.639	125984.9	22	14	11
1	1	0.001	2502.196	317.616	126147.9	23	15	11
1	1	-0.008	2502.191	315.096	126309.5	23	15	11
1	1	0	2249.229	211.393	113193.6	0	16	11
1	1	-0.002	2249.228	218.858	113305.9	0	16	11
1	1	-0.002	2249.227	229.76	113423.7	1	17	11
1	1	-0.002	2249.226	249.617	113551.9	1	17	11
1	1	-0.003	2249.224	278.526	113694.9	2	18	11
1	1	-0.002	2249.223	218.596	113807	2	18	11
1	1	-0.002	2249.222	232.518	113926.3	3	19	11
1	1	-0.002	2249.221	237.626	114048.1	3	19	11
1	1	-0.002	2249.219	214.204	114158	4	20	11
1	1	-0.002	2249.218	213.541	114267.7	4	20	11

1	1	-0.003	2249.217	214.771	114377.9	5	21	11
1	1	-0.004	2249.215	216.371	114488.9	6	22	11
1	1	-0.004	2249.213	194.764	114588.8	6	22	11
1	1	-0.004	2249.211	207.533	114695.3	7	23	11
1	1	-0.004	2249.208	204.122	114800	7	23	11
1	1	0.051	2502.176	193.879	127994.6	8	0	12
1	1	0.048	2502.2	162.765	128078.1	8	0	12
1	1	-0.009	2502.187	293.34	126460.1	0	16	12
1	1	-0.009	2502.183	253.651	126590.2	0	16	12
1	1	-0.008	2502.178	206.853	126696.3	1	17	12
1	1	-0.008	2502.174	157.196	126777	1	17	12
1	1	-0.008	2502.17	99.992	126827.1	2	18	12
1	1	-0.01	2502.165	302.127	126982.2	2	18	12
1	1	-0.009	2502.161	232.028	127101.3	3	19	12
1	1	-0.009	2502.156	202.721	127205.3	3	19	12
1	1	-0.009	2502.152	202.172	127309.2	4	20	12
1	1	-0.009	2502.147	211.764	127417.8	4	20	12
1	1	-0.008	2502.143	179.885	127510.1	5	21	12
1	1	-0.008	2502.139	180.498	127602.7	5	21	12
1	1	-0.006	2502.136	167.398	127688.5	6	22	12
1	1	-0.004	2502.134	172.5	127777.1	6	22	12
1	1	0.003	2502.135	147.964	127853	7	23	12
1	1	0.027	2502.149	83.892	127895.1	7	23	12
1	2	0.068	568.334	26.474	25820.22	8	0	8
1	2	0.089	568.381	32.202	25837.1	8	0	8
1	2	0.081	839.701	23.881	29395.91	8	0	8
1	2	0.08	839.742	21.139	29406.75	8	0	8
1	2	0.549	568.668	183.52	25933.25	9	1	8
1	2	4.033	570.83	378.458	26136.14	9	1	8
1	2	0.266	839.878	110.206	29463.32	9	1	8
1	2	0.54	840.167	254.585	29599.53	9	1	8
1	2	31.901	587.898	362.268	26329.96	10	2	8
1	2	63.998	622.137	395.168	26541.38	10	2	8
1	2	3.257	841.911	294.838	29757.35	10	2	8
1	2	15.857	850.394	300.308	29918.01	10	2	8
1	2	63.698	656.251	360.014	26734.18	11	3	8
1	2	63.123	690.057	284.621	26886.61	11	3	8
1	2	53.55	879.043	375.457	30118.88	11	3	8
1	2	53.632	718.779	156.832	26970.6	12	4	8
1	2	24.482	731.619	89.135	27017.35	12	4	8
1	2	63.937	913.321	415.589	30341.68	12	4	8
1	2	59.157	945.002	414.985	30563.93	12	4	8
1	2	44.174	755.289	242.527	27147.3	13	5	8

1	2	40.042	966.447	403.325	30779.94	13	5	8
1	2	26.058	980.402	388.551	30988.03	13	5	8
1	2	36.057	774.589	326.562	27322.1	14	6	8
1	2	26.999	789.033	331.163	27499.28	14	6	8
1	2	17.676	989.883	395.405	31200.12	14	6	8
1	2	12.661	996.661	374.033	31400.33	14	6	8
1	2	20.116	799.795	312.526	27666.48	15	7	8
1	2	17.456	809.139	340.715	27848.85	15	7	8
1	2	10.774	1002.431	414.081	31622.09	15	7	8
1	2	7.586	1006.493	373.188	31821.96	15	7	8
1	2	12.12	815.63	288.876	28003.56	16	8	8
1	2	11.018	821.525	336.128	28183.39	16	8	8
1	2	4.654	1008.989	261.554	31962.18	16	8	8
1	2	4.314	1011.299	304.416	32125.21	16	8	8
1	2	8.166	825.898	304.191	28346.3	17	9	8
1	2	5.269	828.72	227.764	28468.28	17	9	8
1	2	3.568	1013.21	297.309	32284.43	17	9	8
1	2	2.64	1014.622	246.44	32416.35	17	9	8
1	2	6.057	831.961	324.781	28642.04	18	10	8
1	2	3.854	834.022	226.509	28763.22	18	10	8
1	2	1.916	1015.649	202.833	32524.98	18	10	8
1	2	2.444	835.331	180.489	28859.88	19	11	8
1	2	1.353	836.057	123.733	28926.22	19	11	8
1	2	1.382	1016.389	181.037	32622.03	19	11	8
1	2	1.263	1017.065	214.912	32737.01	19	11	8
1	2	1.023	836.594	64.232	28959.9	20	12	8
1	2	0.803	837.014	54.133	28988.26	20	12	8
1	2	0.759	1017.472	165.027	32825.48	20	12	8
1	2	0.441	1017.708	130.532	32895.32	20	12	8
1	2	0.734	837.407	94.741	29039	21	13	8
1	2	0.343	1017.888	81.131	32937.91	21	13	8
1	2	0.058	1017.919	72.852	32976.89	21	13	8
1	2	0.687	837.768	57.887	29069.34	22	14	8
1	2	0.453	838.005	41.563	29091.12	22	14	8
1	2	0.227	1018.039	77.064	33017.35	22	14	8
1	2	0.098	1018.09	28.57	33032.33	22	14	8
1	2	0.731	838.389	75.48	29130.74	23	15	8
1	2	0.381	838.589	93.982	29180.06	23	15	8
1	2	0.183	1018.186	63.286	33065.49	23	15	8
1	2	0.122	1018.25	45.938	33089.61	23	15	8
1	2	0.164	838.675	26.858	29194.16	0	16	8
1	2	0.227	838.794	39.441	29214.83	0	16	8
1	2	0.098	838.843	17.44	29223.58	1	17	8

1	2	0.161	838.927	28.952	29238.77	1	17	8
1	2	0.166	839.011	28.672	29253.15	2	18	8
1	2	0.156	839.093	30.979	29269.41	2	18	8
1	2	0.15	839.17	32.646	29286.17	3	19	8
1	2	0.129	839.236	20.33	29296.6	3	19	8
1	2	0.118	839.296	25.974	29309.92	4	20	8
1	2	0.112	839.355	24.706	29322.87	4	20	8
1	2	0.105	839.408	16.138	29330.97	5	21	8
1	2	0.115	839.468	28.275	29345.79	5	21	8
1	2	0.102	839.52	26.389	29359.34	6	22	8
1	2	0.1	839.572	17.87	29368.51	6	22	8
1	2	0.089	839.617	15.5	29376.29	7	23	8
1	2	0.083	839.658	14.12	29383.38	7	23	8
1	2	0.057	1018.78	44.031	33439.63	8	0	9
1	2	0.05	1018.806	33.49	33457.17	8	0	9
1	2	0.051	1018.832	31.304	33473.23	9	1	9
1	2	0.044	1018.855	31.693	33489.49	9	1	9
1	2	0.095	1018.903	112.075	33546.96	10	2	9
1	2	0.192	1019.006	235.904	33673.3	10	2	9
1	2	0.709	1019.378	192.922	33774.48	11	3	9
1	2	2.26	1020.563	163.597	33860.27	11	3	9
1	2	5.696	1023.612	180.043	33956.64	12	4	9
1	2	11.861	1029.965	260.951	34096.4	12	4	9
1	2	17.178	1039.169	327.258	34271.75	13	5	9
1	2	23.203	1051.602	420.42	34497.03	13	5	9
1	2	22.054	1063.413	403.539	34713.14	14	6	9
1	2	18.526	1073.335	385.967	34919.85	14	6	9
1	2	15.107	1081.433	414.574	35142.11	15	7	9
1	2	11.442	1087.562	402.301	35357.56	16	8	9
1	2	9.458	1092.521	420.599	35578.14	16	8	9
1	2	6.674	1096.092	350.121	35765.46	17	9	9
1	2	5.667	1099.132	383.841	35971.35	17	9	9
1	2	3.418	1100.924	272.061	36114.03	18	10	9
1	2	1.21	1101.573	128.983	36183.18	18	10	9
1	2	1.4	1102.291	133.723	36251.75	19	11	9
1	2	1.831	1103.251	220.981	36367.58	19	11	9
1	2	1.513	1104.06	208.666	36479.21	20	12	9
1	2	1.177	1104.677	195.172	36581.52	20	12	9
1	2	0.8	1105.096	156.644	36663.67	21	13	9
1	2	0.643	1105.426	85.577	36707.55	21	13	9
1	2	0.476	1105.675	130.035	36775.71	22	14	9
1	2	0.312	1105.835	51.386	36802.07	22	14	9
1	2	0.269	1105.976	39.283	36822.65	23	15	9

1	2	0.203	1106.081	35.978	36841.11	23	15	9
1	2	0.086	1018.295	29.828	33105.23	0	16	9
1	2	0.151	1018.374	59.581	33136.48	0	16	9
1	2	0.065	1018.408	92.018	33184.71	1	17	9
1	2	0.062	1018.44	46.257	33208.97	1	17	9
1	2	0.043	1018.462	22.675	33220.85	2	18	9
1	2	0.064	1018.495	44.729	33243.8	2	18	9
1	2	0.042	1018.517	29.854	33259.47	3	19	9
1	2	0.071	1018.555	44.145	33282.59	3	19	9
1	2	0.057	1018.584	42.636	33304.49	4	20	9
1	2	0.043	1018.606	32.095	33321.3	4	20	9
1	2	0.074	1018.644	49.468	33346.7	5	21	9
1	2	0.048	1018.669	29.959	33362.42	6	22	9
1	2	0.054	1018.697	37.162	33381.47	6	22	9
1	2	0.048	1018.722	28.538	33396.45	7	23	9
1	2	0.055	1018.751	39.24	33417.01	7	23	9
1	2	0.136	1107.404	34.006	37099.45	8	0	10
1	2	0.123	1107.469	28.568	37114.43	8	0	10
1	2	0.249	1107.593	61.073	37145.09	9	1	10
1	2	0.569	1107.892	173.558	37236.21	9	1	10
1	2	3.203	1109.607	272.308	37382.04	10	2	10
1	2	8.099	1113.945	218.527	37499.08	10	2	10
1	2	15.63	1122.133	261.343	37635.99	11	3	10
1	2	28.621	1137.461	407.262	37854.1	11	3	10
1	2	30.536	1153.467	498.109	38115.2	12	4	10
1	2	22.648	1165.596	474.439	38369.29	12	4	10
1	2	17.695	1174.876	520.082	38642.04	13	5	10
1	2	11.787	1181.189	491.911	38905.48	14	6	10
1	2	8.511	1185.749	484.713	39165.21	14	6	10
1	2	7.324	1189.668	483.379	39423.82	15	7	10
1	2	5.627	1192.617	447.145	39658.2	15	7	10
1	2	4.42	1194.982	455.048	39901.65	16	8	10
1	2	3.18	1196.648	388.441	40105.26	16	8	10
1	2	2.217	1197.835	325.092	40279.27	17	9	10
1	2	1.847	1198.803	353.7	40464.67	17	9	10
1	2	1.234	1199.45	291.256	40617.26	18	10	10
1	2	0.696	1199.815	225.87	40735.78	18	10	10
1	2	0.503	1200.078	199.223	40840.26	19	11	10
1	2	0.355	1200.264	209.339	40950.04	19	11	10
1	2	0.138	1200.337	164.446	41036.2	20	12	10
1	2	-0.001	1200.336	120.751	41099.49	20	12	10
1	2	0.064	1200.37	50.382	41125.91	21	13	10
1	2	0.084	1200.413	42.597	41147.77	21	13	10

1	2	0.086	1200.458	39.061	41168.25	22	14	10
1	2	0.068	1200.493	33.461	41185.43	22	14	10
1	2	0.077	1200.532	41.181	41206.55	23	15	10
1	2	0.053	1200.56	31.398	41222.67	23	15	10
1	2	0.247	1106.208	40.847	36862.07	0	16	10
1	2	0.202	1106.312	33.215	36879.1	0	16	10
1	2	0.198	1106.413	31.5	36895.26	1	17	10
1	2	0.178	1106.505	31.451	36911.39	1	17	10
1	2	0.17	1106.592	29.004	36926.27	2	18	10
1	2	0.166	1106.678	28.667	36941.32	2	18	10
1	2	0.153	1106.757	29.874	36956.64	3	19	10
1	2	0.148	1106.834	27.106	36970.84	4	20	10
1	2	0.14	1106.908	29.934	36986.54	4	20	10
1	2	0.137	1106.98	26.629	37000.52	5	21	10
1	2	0.152	1107.059	31.94	37017.26	5	21	10
1	2	0.122	1107.123	28.652	37032.29	6	22	10
1	2	0.139	1107.196	30.624	37048.36	6	22	10
1	2	0.133	1107.264	31.465	37064.49	7	23	10
1	2	0.133	1107.334	33.351	37082	7	23	10
1	2	0.049	1200.988	30.514	41475.23	8	0	11
1	2	0.042	1201.009	29.692	41490.45	8	0	11
1	2	0.076	1201.047	40.824	41510.95	9	1	11
1	2	0.442	1201.275	196.543	41611.89	9	1	11
1	2	3.828	1203.323	421.935	41837.63	10	2	11
1	2	9.452	1208.379	409.227	42056.56	10	2	11
1	2	12.48	1214.921	409.872	42271.4	11	3	11
1	2	11.503	1220.947	401.425	42481.7	11	3	11
1	2	10.801	1226.725	484.877	42741.11	12	4	11
1	2	8.743	1231.408	531.316	43025.66	12	4	11
1	2	6.992	1235.075	550.372	43314.3	13	5	11
1	2	5.385	1237.957	537.945	43602.25	13	5	11
1	2	5.04	1240.602	587.315	43910.43	14	6	11
1	2	4.373	1242.943	566.3	44213.56	15	7	11
1	2	3.88	1244.978	549.223	44501.59	15	7	11
1	2	3.095	1246.634	520.171	44779.89	16	8	11
1	2	2.262	1247.82	493.671	45038.79	16	8	11
1	2	1.647	1248.683	449.993	45274.79	17	9	11
1	2	0.875	1249.142	296.363	45430.21	17	9	11
1	2	0.653	1249.485	268.468	45571.01	18	10	11
1	2	0.456	1249.724	257.57	45706.09	18	10	11
1	2	0.21	1249.833	189.778	45805.57	19	11	11
1	2	0.179	1249.927	201.016	45910.88	19	11	11
1	2	0.122	1249.991	238.36	46035.82	20	12	11

1	2	-0.002	1249.99	152.299	46115.61	20	12	11
1	2	0.031	1250.006	70.975	46152.02	21	13	11
1	2	-0.001	1250.005	137.592	46224.14	21	13	11
1	2	0.002	1250.007	76.315	46263.32	22	14	11
1	2	-0.001	1250.006	122.828	46327.66	22	14	11
1	2	0	1250.006	32.343	46344.26	23	15	11
1	2	0	1250.006	38.08	46363.8	23	15	11
1	2	0.051	1200.586	30.361	41238.25	0	16	11
1	2	0.059	1200.616	36.246	41256.84	0	16	11
1	2	0.066	1200.65	30.847	41272.68	1	17	11
1	2	0.073	1200.688	36.861	41291.6	2	18	11
1	2	0.054	1200.715	32.676	41308.37	2	18	11
1	2	0.05	1200.741	28.806	41323.14	3	19	11
1	2	0.041	1200.762	29.241	41338.14	3	19	11
1	2	0.055	1200.791	29.987	41353.53	4	20	11
1	2	0.049	1200.816	29.712	41368.76	4	20	11
1	2	0.045	1200.839	30.004	41384.17	5	21	11
1	2	0.046	1200.863	28.067	41398.58	5	21	11
1	2	0.05	1200.888	30.677	41414.32	6	22	11
1	2	0.051	1200.915	30.05	41429.73	6	22	11
1	2	0.042	1200.936	28.791	41444.5	7	23	11
1	2	0.052	1200.963	29.332	41459.55	7	23	11
1	2	0.038	1250.117	35.592	46638.93	8	0	12
1	2	0.035	1250.135	48.867	46664	8	0	12
1	2	0	1250.006	39.99	46384.32	0	16	12
1	2	0	1250.005	33.499	46401.51	0	16	12
1	2	0	1250.006	29.84	46416.82	1	17	12
1	2	0.001	1250.006	21.306	46427.5	1	17	12
1	2	0.007	1250.01	39.993	46448.04	2	18	12
1	2	0.007	1250.013	29.387	46463.11	2	18	12
1	2	0.008	1250.018	33.567	46480.34	3	19	12
1	2	0.011	1250.023	31.675	46496.61	3	19	12
1	2	0.015	1250.031	36.469	46515.32	4	20	12
1	2	0.014	1250.039	34.248	46532.89	5	21	12
1	2	0.019	1250.048	32.319	46549.47	5	21	12
1	2	0.02	1250.058	38.685	46569.32	6	22	12
1	2	0.029	1250.073	44.154	46591.98	6	22	12
1	2	0.025	1250.086	32.359	46608.58	7	23	12
1	2	0.022	1250.097	24.102	46620.68	7	23	12
1	3	0.165	42.076	13.892	3128.248	8	0	8
1	3	0.15	42.154	12.108	3134.592	8	0	8
1	3	0.124	62.031	6.568	3574.655	8	0	8
1	3	0.124	62.094	8.445	3578.986	8	0	8

1	3	0.328	42.33	91.62	3183.659	9	1	8
1	3	0.336	42.51	29.283	3199.342	9	1	8
1	3	0.217	62.208	52.786	3606.669	9	1	8
1	3	0.947	43.016	29.205	3214.967	10	2	8
1	3	2.755	44.491	32.327	3232.261	10	2	8
1	3	0.175	62.302	29.1	3622.245	10	2	8
1	3	0.249	62.435	18.231	3631.999	10	2	8
1	3	3.016	46.106	37.648	3252.424	11	3	8
1	3	0.826	62.877	33.844	3650.105	11	3	8
1	3	1.106	63.469	35.049	3668.866	11	3	8
1	3	2.266	47.32	26.647	3266.694	12	4	8
1	3	-0.269	47.179	19.956	3277.16	12	4	8
1	3	1.01	64.011	22.553	3680.964	12	4	8
1	3	0.993	64.542	25.101	3694.393	12	4	8
1	3	1.96	48.23	23.256	3289.628	13	5	8
1	3	1.984	49.292	25.156	3303.101	13	5	8
1	3	0.915	65.032	22.437	3706.409	13	5	8
1	3	0.928	65.529	19.614	3716.919	13	5	8
1	3	1.782	50.245	22.475	3315.125	14	6	8
1	3	1.735	51.173	22.071	3326.933	14	6	8
1	3	0.897	66.011	26.195	3730.969	14	6	8
1	3	0.825	66.452	19.212	3741.253	14	6	8
1	3	1.557	52.007	21.692	3338.544	15	7	8
1	3	1.557	52.839	23.69	3351.218	15	7	8
1	3	0.753	66.855	19.376	3751.63	15	7	8
1	3	0.741	67.252	20.259	3762.485	15	7	8
1	3	1.449	53.615	22.057	3363.031	16	8	8
1	3	1.38	54.354	22.012	3374.807	16	8	8
1	3	0.816	67.689	20.102	3773.251	16	8	8
1	3	0.725	68.078	18.341	3783.079	16	8	8
1	3	1.321	55.061	24.165	3387.756	17	9	8
1	3	1.245	55.728	22.203	3399.64	17	9	8
1	3	0.657	68.429	20.95	3794.292	17	9	8
1	3	1.013	56.27	22.517	3411.687	18	10	8
1	3	1.043	56.828	22.299	3423.617	18	10	8
1	3	0.594	68.747	18.727	3804.322	18	10	8
1	3	0.55	69.042	20.843	3815.491	18	10	8
1	3	0.893	57.307	20.617	3434.67	19	11	8
1	3	0.506	69.313	18.153	3825.213	19	11	8
1	3	0.462	69.56	18.74	3835.239	19	11	8
1	3	0.79	57.721	14.405	3442.225	20	12	8
1	3	0.701	58.089	13.284	3449.192	20	12	8
1	3	0.42	69.785	15.697	3843.654	20	12	8

1	3	0.421	70.006	24.27	3856.369	20	12	8
1	3	0.713	58.47	19.103	3459.412	21	13	8
1	3	0.639	58.805	14.763	3467.154	21	13	8
1	3	0.342	70.189	14.465	3864.124	21	13	8
1	3	0.338	70.366	14.025	3871.471	21	13	8
1	3	0.568	59.103	12.421	3473.665	22	14	8
1	3	0.52	59.376	13.269	3480.616	22	14	8
1	3	0.307	70.527	13.44	3878.527	22	14	8
1	3	0.301	70.685	20.68	3889.372	22	14	8
1	3	0.51	59.65	16.363	3489.403	23	15	8
1	3	0.469	59.891	12.054	3495.587	23	15	8
1	3	0.264	70.823	13.003	3896.184	23	15	8
1	3	0.255	70.957	17.465	3905.353	23	15	8
1	3	0.426	60.114	11.194	3501.461	0	16	8
1	3	0.379	60.313	9.241	3506.305	0	16	8
1	3	0.384	60.506	14.598	3513.632	1	17	8
1	3	0.329	60.678	9.786	3518.764	1	17	8
1	3	0.313	60.835	11.346	3524.46	2	18	8
1	3	0.273	60.978	9.052	3529.207	2	18	8
1	3	0.26	61.112	8.8	3533.724	3	19	8
1	3	0.241	61.235	9.416	3538.555	3	19	8
1	3	0.226	61.351	9.373	3543.364	4	20	8
1	3	0.206	61.459	7.557	3547.327	4	20	8
1	3	0.202	61.561	8.275	3551.481	5	21	8
1	3	0.177	61.654	8.463	3555.915	5	21	8
1	3	0.163	61.737	8.728	3560.395	6	22	8
1	3	0.16	61.82	6.941	3563.96	6	22	8
1	3	0.151	61.896	7.884	3567.917	7	23	8
1	3	0.14	61.966	6.561	3571.208	7	23	8
1	3	0.106	72.269	18.322	4034.708	8	0	9
1	3	0.088	72.315	13.792	4041.938	8	0	9
1	3	0.09	72.362	15.225	4049.745	9	1	9
1	3	0.105	72.415	30.26	4065.27	9	1	9
1	3	0.083	72.459	18.879	4075.16	10	2	9
1	3	0.207	72.57	114.761	4136.685	10	2	9
1	3	0.121	72.633	34.368	4154.69	11	3	9
1	3	0.191	72.733	45.057	4178.333	11	3	9
1	3	0.293	72.89	43.973	4201.859	12	4	9
1	3	0.146	72.969	45.924	4226.467	12	4	9
1	3	-0.088	72.921	31.408	4243.288	13	5	9
1	3	0.189	73.023	31.674	4260.269	14	6	9
1	3	0.27	73.167	30.895	4276.815	14	6	9
1	3	0.243	73.297	27.638	4291.625	15	7	9

1	3	0.249	73.43	26.679	4305.913	15	7	9
1	3	0.3	73.591	27.21	4320.493	16	8	9
1	3	0.272	73.734	23.705	4332.918	16	8	9
1	3	0.362	73.927	77.184	4374.211	17	9	9
1	3	0.246	74.059	6.823	4377.875	17	9	9
1	3	0.262	74.197	32.094	4394.688	18	10	9
1	3	0.263	74.335	19.793	4405.08	18	10	9
1	3	0.223	74.452	24.806	4418.082	19	11	9
1	3	0.22	74.567	29.005	4433.278	19	11	9
1	3	0.216	74.683	24.124	4446.184	20	12	9
1	3	0.2	74.787	23.33	4458.419	20	12	9
1	3	0.151	74.865	18.793	4468.062	21	13	9
1	3	0.18	74.959	22.98	4480.107	21	13	9
1	3	0.155	75.039	16.261	4488.445	22	14	9
1	3	0.127	75.104	14.085	4495.672	22	14	9
1	3	0.125	75.17	12.2	4502.066	23	15	9
1	3	0.235	71.08	16.159	3913.823	0	16	9
1	3	0.227	71.202	20.277	3924.683	0	16	9
1	3	0.214	71.311	15.34	3932.549	1	17	9
1	3	0.206	71.419	18.95	3942.488	1	17	9
1	3	0.179	71.513	13.962	3949.806	2	18	9
1	3	0.177	71.604	14.755	3957.377	3	19	9
1	3	0.16	71.688	13.507	3964.464	3	19	9
1	3	0.148	71.765	13.76	3971.677	4	20	9
1	3	0.144	71.839	13.743	3978.731	4	20	9
1	3	0.136	71.91	14.067	3986.105	5	21	9
1	3	0.127	71.975	13.375	3992.971	5	21	9
1	3	0.114	72.035	13.786	4000.201	6	22	9
1	3	0.125	72.099	14.227	4007.5	6	22	9
1	3	0.109	72.156	20.033	4018.012	7	23	9
1	3	0.112	72.215	13.91	4025.303	7	23	9
1	3	0.071	75.837	10.861	4608.152	8	0	10
1	3	0.057	75.867	9.641	4613.208	8	0	10
1	3	0.088	75.911	35.951	4631.253	9	1	10
1	3	0.125	75.978	50.351	4658.233	9	1	10
1	3	0.186	76.078	33.995	4676.439	10	2	10
1	3	-0.116	76.015	22.407	4688.439	10	2	10
1	3	-0.086	75.97	45.673	4712.367	11	3	10
1	3	0.154	76.053	35.442	4731.348	11	3	10
1	3	0.249	76.183	31.88	4748.067	12	4	10
1	3	0.281	76.334	31.237	4764.796	13	5	10
1	3	0.307	76.495	38.982	4785.24	13	5	10
1	3	0.287	76.648	38.325	4805.766	14	6	10

1	3	0.235	76.775	32.857	4823.362	14	6	10
1	3	0.256	76.911	40.775	4845.177	15	7	10
1	3	0.25	77.042	45.78	4869.173	15	7	10
1	3	0.249	77.175	40.831	4891.018	16	8	10
1	3	0.279	77.322	46.844	4915.584	16	8	10
1	3	0.29	77.477	43.7	4938.976	17	9	10
1	3	0.244	77.604	43.939	4961.995	17	9	10
1	3	0.287	77.755	50.091	4988.251	18	10	10
1	3	0.266	77.894	49.872	5014.42	18	10	10
1	3	0.246	78.023	43.551	5037.248	19	11	10
1	3	0.237	78.147	43.184	5059.896	19	11	10
1	3	0.198	78.251	35.094	5078.281	20	12	10
1	3	0.179	78.343	30.289	5093.821	20	12	10
1	3	0.182	78.439	34.289	5111.804	21	13	10
1	3	0.18	78.531	38.545	5131.58	22	14	10
1	3	0.15	78.61	29.665	5147.138	22	14	10
1	3	0.143	78.683	27.318	5161.161	23	15	10
1	3	0.119	78.744	26.118	5174.561	23	15	10
1	3	0.112	75.227	14.761	4509.636	0	16	10
1	3	0.109	75.283	15.003	4517.333	0	16	10
1	3	0.096	75.332	11.788	4523.381	1	17	10
1	3	0.092	75.379	12.469	4529.774	1	17	10
1	3	0.083	75.421	13.515	4536.708	2	18	10
1	3	0.085	75.465	13.037	4543.397	2	18	10
1	3	0.072	75.503	10.656	4548.989	3	19	10
1	3	0.073	75.54	11.265	4554.769	3	19	10
1	3	0.074	75.579	11.36	4560.723	4	20	10
1	3	0.069	75.615	11.135	4566.563	4	20	10
1	3	0.061	75.647	11.51	4572.603	5	21	10
1	3	0.063	75.68	11.715	4578.743	5	21	10
1	3	0.06	75.711	10.524	4584.262	6	22	10
1	3	0.063	75.744	11.366	4590.223	6	22	10
1	3	0.054	75.772	11.77	4596.262	7	23	10
1	3	0.054	75.8	12.029	4602.573	7	23	10
1	3	0.049	79.424	12.298	5341.226	8	0	11
1	3	0.048	79.449	11.05	5346.895	8	0	11
1	3	0.068	79.483	25.929	5359.91	9	1	11
1	3	0.103	79.537	33.466	5377.461	9	1	11
1	3	0.169	79.627	25.875	5391.305	10	2	11
1	3	0.128	79.696	26.716	5405.598	10	2	11
1	3	-0.296	79.541	20.175	5416.173	11	3	11
1	3	0.003	79.542	59.691	5447.444	11	3	11
1	3	0.304	79.705	49.369	5473.871	12	4	11

1	3	0.252	79.84	38.471	5494.463	13	5	11
1	3	0.135	79.911	30.445	5510.438	13	5	11
1	3	0.38	80.115	40.123	5531.916	14	6	11
1	3	0.454	80.353	46.057	5556.083	14	6	11
1	3	0.346	80.538	39.716	5577.342	15	7	11
1	3	0.377	80.736	37.977	5597.249	15	7	11
1	3	0.511	81.009	48.555	5623.252	16	8	11
1	3	0.3	81.166	32.186	5640.132	16	8	11
1	3	0.59	81.475	55.545	5669.231	17	9	11
1	3	0.421	81.696	44.354	5692.493	17	9	11
1	3	0.393	81.902	39.21	5713.067	18	10	11
1	3	0.43	82.127	46.156	5737.273	18	10	11
1	3	0.35	82.311	38.681	5757.538	19	11	11
1	3	0.378	82.509	42.545	5779.838	19	11	11
1	3	0.322	82.678	38.027	5799.76	20	12	11
1	3	0.319	82.841	32.606	5816.489	20	12	11
1	3	0.272	82.984	34.16	5834.385	21	13	11
1	3	0.252	83.113	26.228	5847.842	21	13	11
1	3	0.231	83.234	29.586	5863.358	22	14	11
1	3	0.222	83.348	22.242	5874.77	22	14	11
1	3	0.206	83.454	22.381	5886.252	23	15	11
1	3	0.204	83.559	22.512	5897.808	23	15	11
1	3	0.124	78.808	27.404	5188.628	0	16	11
1	3	0.106	78.862	24.134	5201.011	0	16	11
1	3	0.101	78.914	22.008	5212.302	1	17	11
1	3	0.106	78.968	25.605	5225.446	1	17	11
1	3	0.086	79.012	21.592	5236.53	2	18	11
1	3	0.087	79.057	22.032	5247.833	2	18	11
1	3	0.071	79.093	19.332	5257.752	3	19	11
1	3	0.098	79.143	21.907	5268.985	3	19	11
1	3	0.069	79.179	19.588	5279.035	4	20	11
1	3	0.074	79.216	16.896	5287.704	4	20	11
1	3	0.058	79.246	16.374	5296.109	5	21	11
1	3	0.072	79.283	15.932	5304.288	5	21	11
1	3	0.052	79.31	16.229	5312.614	6	22	11
1	3	0.063	79.342	16.599	5321.13	6	22	11
1	3	0.058	79.372	13.824	5328.219	7	23	11
1	3	0.052	79.399	13.033	5334.913	7	23	11
1	3	0.104	84.634	14.189	6026.303	8	0	12
1	3	0.103	84.687	16.823	6034.93	8	0	12
1	3	0.178	83.65	18.455	5907.277	0	16	12
1	3	0.17	83.737	18.286	5916.653	1	17	12
1	3	0.159	83.819	16.424	5925.08	1	17	12

1	3	0.167	83.902	19.995	5935.116	2	18	12
1	3	0.147	83.977	17.359	5944.027	2	18	12
1	3	0.139	84.049	16.299	5952.389	3	19	12
1	3	0.136	84.119	17.536	5961.391	3	19	12
1	3	0.127	84.184	14.624	5968.897	4	20	12
1	3	0.121	84.246	14.223	5976.195	4	20	12
1	3	0.122	84.309	14.419	5983.592	5	21	12
1	3	0.111	84.366	14.216	5990.882	5	21	12
1	3	0.11	84.423	14.594	5998.369	6	22	12
1	3	0.103	84.475	12.878	6004.98	6	22	12
1	3	0.102	84.528	12.733	6011.513	7	23	12
1	3	0.107	84.581	14.962	6019.023	7	23	12
1	4	0.973	626.782	119.292	19551.41	8	0	8
1	4	1.255	627.44	184.916	19648.28	8	0	8
1	4	-0.026	751.983	101.315	24827.61	8	0	8
1	4	-0.025	751.97	153.451	24908.09	8	0	8
1	4	3.213	629.16	331.393	19825.76	9	1	8
1	4	8.721	633.831	224.734	19946.12	9	1	8
1	4	0	751.971	109.713	24964.38	9	1	8
1	4	0.106	752.026	326.391	25135.55	9	1	8
1	4	20.982	645.056	197.595	20051.83	10	2	8
1	4	1.255	752.697	276.844	25283.66	10	2	8
1	4	5.027	755.387	237.458	25410.7	10	2	8
1	4	40.779	666.884	239.537	20180.05	11	3	8
1	4	50.089	693.71	302.685	20342.16	11	3	8
1	4	13.003	762.347	226.02	25531.69	11	3	8
1	4	20.475	773.301	229.668	25654.56	11	3	8
1	4	32.821	711.287	245.776	20473.79	12	4	8
1	4	22.585	723.132	275.528	20618.29	12	4	8
1	4	25.044	786.735	262.569	25795.4	12	4	8
1	4	23.017	799.049	295.918	25953.71	12	4	8
1	4	15.498	731.436	341.819	20801.44	13	5	8
1	4	10.729	737.182	363.445	20996.09	13	5	8
1	4	16.834	808.064	278.339	26102.78	13	5	8
1	4	10.816	813.863	258.016	26241.11	13	5	8
1	4	7.723	741.314	369.527	21193.79	14	6	8
1	4	5.286	744.143	324.657	21367.57	14	6	8
1	4	6.582	817.393	277.826	26390.13	14	6	8
1	4	4.777	819.949	355.297	26580.21	14	6	8
1	4	4.309	746.449	403.254	21583.31	15	7	8
1	4	3.132	748.124	389.736	21791.81	15	7	8
1	4	2.906	821.506	304.475	26743.28	15	7	8
1	4	2.433	749.427	392.367	22001.95	16	8	8

1	4	1.911	750.45	344.426	22186.22	16	8	8
1	4	1.785	822.462	287.894	26897.54	16	8	8
1	4	1.346	823.183	298.656	27057.49	16	8	8
1	4	1.263	751.127	314.1	22354.61	17	9	8
1	4	0.766	751.537	264.988	22496.38	17	9	8
1	4	1.334	823.899	381.32	27261.92	17	9	8
1	4	0.999	824.433	307.188	27426.26	17	9	8
1	4	0.822	751.976	308.71	22661.54	18	10	8
1	4	0.805	824.864	287.601	27580.29	18	10	8
1	4	0.605	825.188	268.496	27724.16	18	10	8
1	4	0.684	752.343	308.756	22826.81	19	11	8
1	4	0.395	752.555	263.685	22968.1	19	11	8
1	4	0.516	825.465	283.244	27875.85	19	11	8
1	4	0.332	825.643	274.064	28022.55	19	11	8
1	4	0.205	752.662	235.581	23091.65	20	12	8
1	4	0.023	752.674	223.401	23208.81	20	12	8
1	4	0.254	825.778	293.109	28179.61	20	12	8
1	4	0.131	825.847	272.763	28322.58	20	12	8
1	4	-0.04	752.653	263.578	23349.9	21	13	8
1	4	-0.12	752.59	226.75	23468.81	21	13	8
1	4	-0.023	825.835	223.265	28442.21	21	13	8
1	4	-0.061	825.803	206.874	28550.65	21	13	8
1	4	-0.111	752.532	203.231	23575.29	22	14	8
1	4	-0.103	752.478	221.042	23691.15	22	14	8
1	4	-0.058	825.772	217.029	28664.53	22	14	8
1	4	-0.054	825.744	195.298	28766.95	22	14	8
1	4	-0.092	752.429	145.795	23769.39	23	15	8
1	4	-0.089	752.383	141.075	23841.77	23	15	8
1	4	-0.05	825.718	178.705	28860.63	23	15	8
1	4	-0.047	825.693	162.043	28945.7	23	15	8
1	4	-0.08	752.341	176.873	23934.63	0	16	8
1	4	-0.073	752.303	114.769	23994.76	0	16	8
1	4	-0.071	752.267	85.533	24037.69	1	17	8
1	4	-0.063	752.234	144.733	24113.59	1	17	8
1	4	-0.061	752.203	105.382	24166.49	2	18	8
1	4	-0.054	752.175	151.513	24245.95	2	18	8
1	4	-0.051	752.149	112.356	24303.66	3	19	8
1	4	-0.048	752.125	153.726	24382.49	3	19	8
1	4	-0.044	752.102	112.196	24440.05	4	20	8
1	4	-0.04	752.081	146.321	24516.79	4	20	8
1	4	-0.039	752.062	95.789	24564.87	5	21	8
1	4	-0.035	752.043	119.279	24627.42	5	21	8
1	4	-0.033	752.027	107.553	24682.6	6	22	8

1	4	-0.031	752.011	133.139	24750.94	7	23	8
1	4	-0.029	751.996	51.43	24776.76	7	23	8
1	4	0.066	825.651	114.68	30102.87	8	0	9
1	4	0.088	825.697	150.247	30181.63	8	0	9
1	4	0.046	825.721	70.786	30217.94	9	1	9
1	4	0.188	825.817	243.145	30342.62	9	1	9
1	4	0.18	825.911	259.896	30478.85	10	2	9
1	4	0.368	826.109	142.868	30555.4	10	2	9
1	4	1.382	826.833	77.376	30595.96	11	3	9
1	4	3.555	828.697	189.159	30695.17	11	3	9
1	4	4.466	831.087	212.092	30808.63	12	4	9
1	4	3.932	833.194	219.417	30926.27	13	5	9
1	4	3.686	835.168	253.895	31062.24	13	5	9
1	4	2.874	836.708	301.849	31223.98	14	6	9
1	4	1.653	837.594	241.747	31353.45	14	6	9
1	4	0.584	837.907	206.105	31463.89	15	7	9
1	4	0.055	837.936	181.823	31561.27	15	7	9
1	4	-0.033	837.919	106.079	31618.14	16	8	9
1	4	0.063	837.952	149.728	31696.58	16	8	9
1	4	-0.04	837.931	211.888	31810	17	9	9
1	4	-0.037	837.911	102.899	31865.22	17	9	9
1	4	-0.038	837.891	102.258	31918.82	18	10	9
1	4	-0.039	837.87	91.505	31966.84	18	10	9
1	4	-0.037	837.851	55.738	31996.05	19	11	9
1	4	-0.036	837.832	81.037	32038.51	19	11	9
1	4	-0.033	837.814	61.592	32071.48	20	12	9
1	4	-0.032	837.797	48.32	32096.82	21	13	9
1	4	-0.031	837.782	23.873	32109.07	21	13	9
1	4	-0.029	837.767	34.804	32127.3	22	14	9
1	4	-0.028	837.752	29.268	32142.31	22	14	9
1	4	-0.013	837.745	115.786	32201.69	23	15	9
1	4	-0.025	837.732	128.693	32269.14	23	15	9
1	4	-0.044	825.67	158.637	29028.8	0	16	9
1	4	-0.04	825.648	152.673	29110.57	1	17	9
1	4	-0.039	825.629	116.35	29170.26	1	17	9
1	4	-0.036	825.61	169.779	29259.3	2	18	9
1	4	-0.033	825.592	142.203	29333.8	2	18	9
1	4	-0.032	825.576	107.22	29388.81	3	19	9
1	4	-0.03	825.56	159.017	29472.29	3	19	9
1	4	-0.028	825.546	139.937	29545.61	4	20	9
1	4	-0.022	825.534	114.428	29604.35	4	20	9
1	4	-0.023	825.522	148.675	29682.28	5	21	9
1	4	0.013	825.529	113.422	29740.5	5	21	9

1	4	0.008	825.533	156.565	29822.61	6	22	9
1	4	0.048	825.557	123.302	29885.87	6	22	9
1	4	0.049	825.583	157.812	29968.72	7	23	9
1	4	0.065	825.617	143.705	30044	7	23	9
1	4	0.029	837.615	85.603	33239.79	8	0	10
1	4	-0.006	837.612	68.702	33275.82	8	0	10
1	4	0.054	837.639	126.315	33339.23	9	1	10
1	4	0.199	837.746	199.265	33446.05	9	1	10
1	4	1.23	838.404	157.933	33530.59	10	2	10
1	4	1.631	839.277	119.775	33594.74	11	3	10
1	4	1.343	839.981	98.476	33646.36	11	3	10
1	4	0.836	840.429	98.075	33698.88	12	4	10
1	4	0.894	840.898	92.501	33747.39	12	4	10
1	4	0.59	841.214	124.26	33813.94	13	5	10
1	4	0.633	841.546	95.843	33864.2	13	5	10
1	4	0.566	841.849	201.536	33972.14	14	6	10
1	4	0.047	841.874	169.134	34062.72	14	6	10
1	4	-0.033	841.856	126.491	34130.39	15	7	10
1	4	-0.035	841.838	158.236	34213.29	15	7	10
1	4	-0.034	841.82	147.13	34292.04	16	8	10
1	4	-0.035	841.801	44.314	34315.29	16	8	10
1	4	-0.037	841.782	100.293	34368.94	17	9	10
1	4	-0.036	841.763	42.311	34391.12	17	9	10
1	4	-0.037	841.743	23.306	34403.33	18	10	10
1	4	-0.037	841.724	58.523	34434.06	18	10	10
1	4	-0.036	841.705	24.705	34447	19	11	10
1	4	-0.035	841.687	24.69	34459.95	20	12	10
1	4	-0.033	841.669	36.836	34479.26	20	12	10
1	4	0.004	841.671	97.219	34529.16	21	13	10
1	4	-0.03	841.655	139.978	34602.54	21	13	10
1	4	-0.029	841.64	62.241	34634.45	22	14	10
1	4	-0.027	841.626	54.859	34663.22	22	14	10
1	4	-0.025	841.613	33.174	34680.26	23	15	10
1	4	-0.023	841.601	11.925	34686.37	23	15	10
1	4	-0.024	837.72	42.565	32290.98	0	16	10
1	4	-0.022	837.708	25.731	32304.18	0	16	10
1	4	-0.021	837.698	24.584	32316.79	1	17	10
1	4	-0.02	837.687	23.57	32328.88	1	17	10
1	4	-0.019	837.678	41.443	32350.13	2	18	10
1	4	-0.008	837.674	67.258	32384.64	2	18	10
1	4	-0.017	837.665	91.739	32432.8	3	19	10
1	4	-0.015	837.657	129.211	32499.06	3	19	10
1	4	-0.017	837.648	206.776	32607.44	4	20	10

1	4	-0.016	837.64	195.038	32709.73	4	20	10
1	4	-0.015	837.632	193.602	32811.37	5	21	10
1	4	-0.015	837.624	188.012	32909.87	5	21	10
1	4	-0.014	837.617	174.963	33001.63	6	22	10
1	4	-0.013	837.61	137.537	33073.75	6	22	10
1	4	-0.007	837.606	104.463	33127.35	7	23	10
1	4	-0.011	837.6	130.487	33195.85	7	23	10
1	4	0.024	841.575	18.505	34843.13	8	0	11
1	4	0.017	841.584	19.22	34852.99	8	0	11
1	4	0.035	841.601	39.798	34872.97	9	1	11
1	4	0.258	841.737	164.449	34959.21	9	1	11
1	4	0.549	842.031	157.299	35043.37	10	2	11
1	4	0.309	842.196	77.292	35084.74	10	2	11
1	4	-0.241	842.069	33.14	35102.11	11	3	11
1	4	0.367	842.261	92.933	35150.82	12	4	11
1	4	0.396	842.473	189.973	35252.45	12	4	11
1	4	-0.019	842.463	83.157	35296.99	13	5	11
1	4	0.252	842.595	117.71	35358.72	13	5	11
1	4	-0.028	842.58	124.898	35425.61	14	6	11
1	4	0.016	842.589	74.743	35464.81	14	6	11
1	4	-0.041	842.567	125.416	35531.98	15	7	11
1	4	-0.044	842.544	81.308	35574.58	15	7	11
1	4	-0.051	842.517	91.231	35623.44	16	8	11
1	4	-0.049	842.491	43.153	35646.07	16	8	11
1	4	-0.058	842.461	42.654	35668.43	17	9	11
1	4	-0.057	842.431	20.355	35679.11	17	9	11
1	4	-0.057	842.401	24.262	35691.83	18	10	11
1	4	-0.057	842.371	21.394	35703.05	18	10	11
1	4	-0.035	842.353	27.454	35717.44	19	11	11
1	4	-0.022	842.341	19.119	35727.45	19	11	11
1	4	-0.016	842.333	17.741	35736.75	20	12	11
1	4	-0.007	842.329	16.926	35745.43	20	12	11
1	4	-0.008	842.325	15.984	35753.81	21	13	11
1	4	-0.003	842.323	15.099	35761.55	22	14	11
1	4	0.002	842.324	14.175	35768.99	22	14	11
1	4	0.005	842.327	13.653	35775.99	23	15	11
1	4	0.013	842.333	14.361	35783.36	23	15	11
1	4	-0.022	841.59	12.691	34692.89	0	16	11
1	4	-0.02	841.579	11.234	34698.65	0	16	11
1	4	-0.019	841.57	10.526	34704.05	1	17	11
1	4	-0.018	841.561	10.431	34709.41	1	17	11
1	4	-0.017	841.552	10.34	34714.71	2	18	11
1	4	-0.016	841.544	19.86	34724.9	2	18	11

1	4	-0.014	841.537	10.497	34730.29	3	19	11
1	4	-0.014	841.529	9.91	34735.37	3	19	11
1	4	-0.012	841.523	10.533	34740.77	4	20	11
1	4	-0.006	841.52	26.697	34754.47	4	20	11
1	4	0.008	841.524	26.634	34768.14	5	21	11
1	4	0.017	841.533	34.365	34785.79	5	21	11
1	4	0.007	841.537	17.987	34795.01	6	22	11
1	4	0.016	841.545	24.203	34807.43	6	22	11
1	4	0.019	841.555	28.548	34822.08	7	23	11
1	4	0.016	841.563	22.513	34833.63	7	23	11
1	4	0.042	842.591	5.694	35867.62	8	0	12
1	4	0.035	842.609	5.938	35870.67	8	0	12
1	4	0.01	842.339	11.877	35789.46	0	16	12
1	4	0.024	842.351	14.12	35796.7	0	16	12
1	4	0.022	842.362	12.216	35802.97	1	17	12
1	4	0.023	842.374	12.335	35809.3	1	17	12
1	4	0.022	842.385	11.053	35814.84	2	18	12
1	4	0.026	842.398	11.24	35820.61	2	18	12
1	4	0.026	842.412	9.778	35825.63	3	19	12
1	4	0.028	842.426	9.2	35830.35	3	19	12
1	4	0.028	842.44	9.912	35835.44	4	20	12
1	4	0.034	842.458	8.433	35839.77	4	20	12
1	4	0.033	842.475	8.676	35844.22	5	21	12
1	4	0.032	842.491	8.562	35848.61	5	21	12
1	4	0.034	842.509	7.689	35852.56	6	22	12
1	4	0.037	842.528	6.818	35856.06	6	22	12
1	4	0.036	842.546	8.304	35860.32	7	23	12
1	4	0.048	842.57	8.728	35864.7	7	23	12
2	1	0.265	668.195	480.159	63777.92	8	0	8
2	1	0.185	668.295	313.219	63945.58	8	0	8
2	1	0.158	726.645	270.408	76602.53	8	0	8
2	1	2.431	727.947	591.191	76919.15	8	0	8
2	1	0.685	668.661	555.7	64242.88	9	1	8
2	1	3.852	670.726	368.275	64440.32	9	1	8
2	1	25.43	741.559	683.296	77284.9	9	1	8
2	1	52.947	769.886	709.038	77664.23	9	1	8
2	1	9.753	675.946	460.698	64686.92	10	2	8
2	1	14.192	683.543	610.179	65013.53	10	2	8
2	1	64.656	804.477	717.17	78047.92	10	2	8
2	1	64.604	839.112	759.557	78455.13	10	2	8
2	1	16.904	692.596	797.697	65440.74	11	3	8
2	1	15.94	701.128	879.13	65911.32	11	3	8
2	1	65.026	873.937	934.855	78955.8	11	3	8

2	1	12.254	707.691	936.616	66412.93	12	4	8
2	1	59.341	905.685	994.771	79488	12	4	8
2	1	42.124	928.221	1035.039	80041.74	12	4	8
2	1	8.841	712.424	978.358	66936.63	13	5	8
2	1	6.426	715.865	1075.694	67512.72	13	5	8
2	1	29.862	944.529	1113.419	80649.79	13	5	8
2	1	19.241	954.844	1057.508	81216.73	13	5	8
2	1	4.092	718.054	878.2	67982.55	14	6	8
2	1	2.656	719.477	791.521	68406.68	14	6	8
2	1	11.212	960.843	986.549	81744.54	14	6	8
2	1	7.021	964.599	1038.46	82300.12	14	6	8
2	1	1.565	720.316	590.517	68723.27	15	7	8
2	1	0.963	720.831	507.037	68994.53	15	7	8
2	1	4.479	966.996	976.189	82822.65	15	7	8
2	1	3.232	968.728	1063.919	83392.73	15	7	8
2	1	1.253	721.516	760.175	69410.09	16	8	8
2	1	1.284	722.204	862.562	69872.28	16	8	8
2	1	2.161	969.884	887.572	83867.59	16	8	8
2	1	1.561	970.72	724.866	84255.59	16	8	8
2	1	1.293	722.896	871.382	70338.71	17	9	8
2	1	1.363	723.625	934.73	70838.79	17	9	8
2	1	1.62	971.586	699.281	84629.71	17	9	8
2	1	1.694	972.494	739.627	85025.82	17	9	8
2	1	1.149	724.24	799.022	71266.27	18	10	8
2	1	0.963	724.755	730.919	71657.3	18	10	8
2	1	1.304	973.192	629.375	85362.54	18	10	8
2	1	0.874	725.223	764.452	72066.5	19	11	8
2	1	0.988	973.72	590.265	85678.33	19	11	8
2	1	0.494	973.985	434.039	85910.78	19	11	8
2	1	0.564	725.525	640.617	72409.23	20	12	8
2	1	0.348	725.711	526.089	72690.69	20	12	8
2	1	0.228	974.107	356.016	86101.65	20	12	8
2	1	0.213	974.221	473.181	86354.8	20	12	8
2	1	0.286	725.864	596.326	73010.05	21	13	8
2	1	0.181	725.96	538.128	73298.55	21	13	8
2	1	0.092	974.27	464.732	86603.69	21	13	8
2	1	0.011	974.276	446.278	86842.45	21	13	8
2	1	0.109	726.019	484.773	73558.18	22	14	8
2	1	0.047	726.044	362.323	73752.33	22	14	8
2	1	-0.004	974.274	426.485	87070.85	22	14	8
2	1	-0.005	974.272	463.157	87318.64	22	14	8
2	1	0.022	726.056	306.552	73916.5	23	15	8
2	1	0.031	726.072	347.757	74102.55	23	15	8

2	1	-0.004	974.269	408.575	87537.45	23	15	8
2	1	-0.004	974.267	378.393	87740	23	15	8
2	1	0.019	726.082	275.796	74250.25	0	16	8
2	1	0.015	726.091	241.986	74379.71	0	16	8
2	1	0.047	726.116	352.285	74568.38	1	17	8
2	1	0.04	726.137	313.32	74736.09	1	17	8
2	1	0.043	726.16	291.686	74892.15	2	18	8
2	1	0.059	726.192	338.648	75073.33	2	18	8
2	1	0.057	726.222	335.537	75252.93	3	19	8
2	1	0.061	726.255	320.544	75424.42	4	20	8
2	1	0.06	726.288	309.036	75590.02	4	20	8
2	1	0.064	726.322	295.014	75748.18	5	21	8
2	1	0.071	726.36	298.946	75908.12	5	21	8
2	1	0.062	726.394	225.295	76028.65	6	22	8
2	1	0.067	726.429	220.34	76146.66	6	22	8
2	1	0.11	726.488	290.776	76302.3	7	23	8
2	1	0.135	726.56	290.327	76457.71	7	23	8
2	1	0.045	974.315	170.392	90138.04	8	0	9
2	1	0.316	974.48	367.939	90330.9	8	0	9
2	1	3.016	976.096	486.45	90591.55	9	1	9
2	1	6.972	979.826	433.197	90823.31	10	2	9
2	1	9.574	984.951	543.135	91114.04	10	2	9
2	1	6.717	988.548	526.009	91395.74	11	3	9
2	1	5.775	991.707	702.307	91779.87	11	3	9
2	1	4.853	994.305	828.686	92223.67	12	4	9
2	1	4.302	996.607	953.285	92733.68	12	4	9
2	1	3.464	998.499	914.671	93233.2	13	5	9
2	1	2.863	1000.034	946.412	93740.58	13	5	9
2	1	2.549	1001.4	972.721	94261.8	14	6	9
2	1	2.224	1002.591	1019.327	94807.7	14	6	9
2	1	1.904	1003.609	1028.81	95358.12	15	7	9
2	1	1.538	1004.45	1119.05	95969.87	15	7	9
2	1	1.033	1005.003	941.308	96473.47	16	8	9
2	1	0.699	1005.377	830.445	96917.98	17	9	9
2	1	0.682	1005.742	931.577	97417.16	17	9	9
2	1	0.521	1006.021	772.602	97830.5	18	10	9
2	1	0.392	1006.231	689.7	98199.68	18	10	9
2	1	0.351	1006.418	676.614	98561.66	19	11	9
2	1	0.289	1006.573	650.121	98909.48	19	11	9
2	1	0.21	1006.686	537.737	99197.31	20	12	9
2	1	0.09	1006.734	350.945	99385.16	20	12	9
2	1	0.14	1006.809	613.487	99713.55	21	13	9
2	1	0.104	1006.864	537.259	100001	21	13	9

2	1	0.063	1006.898	439.532	100236.1	22	14	9
2	1	0.069	1006.935	478.755	100492.4	22	14	9
2	1	0.052	1006.963	429.69	100722.6	23	15	9
2	1	0.042	1006.985	330.71	100899.6	23	15	9
2	1	-0.004	974.265	328.446	87915.72	0	16	9
2	1	-0.004	974.263	315.699	88084.62	0	16	9
2	1	-0.004	974.261	335.375	88264.13	1	17	9
2	1	-0.004	974.259	327.062	88439.11	1	17	9
2	1	-0.004	974.257	312.866	88606.49	2	18	9
2	1	-0.004	974.255	307.851	88771.53	3	19	9
2	1	-0.004	974.252	274.081	88918.32	3	19	9
2	1	-0.004	974.25	271.6	89063.63	4	20	9
2	1	-0.005	974.248	270.979	89208.6	4	20	9
2	1	-0.005	974.245	269.804	89352.95	5	21	9
2	1	-0.005	974.242	248.86	89486.23	5	21	9
2	1	-0.003	974.241	253.79	89622.15	6	22	9
2	1	0.007	974.245	262.316	89762.48	6	22	9
2	1	0.031	974.261	271.729	89908.01	7	23	9
2	1	0.055	974.291	259.034	90046.88	7	23	9
2	1	0.447	1007.645	414.828	103542.6	8	0	10
2	1	8.297	1012.084	546.783	103835.2	9	1	10
2	1	22.68	1024.242	689.943	104205	9	1	10
2	1	25.199	1037.724	871.618	104671.4	10	2	10
2	1	18.52	1047.632	959.582	105184.7	10	2	10
2	1	12.494	1054.324	1023.451	105732.8	11	3	10
2	1	9.004	1059.146	1119.829	106332.6	11	3	10
2	1	6.968	1062.877	1228.298	106990.4	12	4	10
2	1	5.485	1065.812	1227.45	107647.1	12	4	10
2	1	4.352	1068.142	1227.135	108304.3	13	5	10
2	1	3.825	1070.193	1266.702	108983.4	13	5	10
2	1	3.144	1071.878	1229.82	109642.4	14	6	10
2	1	2.342	1073.132	1199.147	110284.6	14	6	10
2	1	1.555	1073.965	1101.49	110874.5	15	7	10
2	1	1.027	1074.515	986.295	111403	16	8	10
2	1	0.714	1074.898	898.633	111884.2	16	8	10
2	1	0.412	1075.119	764.765	112293.8	17	9	10
2	1	0.261	1075.259	690.385	112663.5	17	9	10
2	1	0.226	1075.38	687.516	113031.7	18	10	10
2	1	0.151	1075.461	678.084	113395.3	18	10	10
2	1	0.126	1075.528	661.927	113749.6	19	11	10
2	1	0.049	1075.554	533.207	114035	19	11	10
2	1	0.011	1075.56	480.796	114292.5	20	12	10
2	1	-0.004	1075.558	483.868	114551.5	20	12	10

2	1	-0.007	1075.555	490.406	114814.1	21	13	10
2	1	-0.015	1075.547	487.311	115075	21	13	10
2	1	-0.019	1075.536	467.016	115325.1	22	14	10
2	1	-0.019	1075.526	433.249	115557.2	22	14	10
2	1	-0.014	1075.519	430.79	115787.7	23	15	10
2	1	0.038	1007.006	297.2	101058.6	0	16	10
2	1	0.057	1007.037	435.036	101292.1	1	17	10
2	1	0.05	1007.063	367.549	101489	1	17	10
2	1	0.048	1007.089	366.436	101685.2	2	18	10
2	1	0.047	1007.114	330.831	101862.2	2	18	10
2	1	0.045	1007.138	315.148	102031	3	19	10
2	1	0.048	1007.164	323.57	102204.3	3	19	10
2	1	0.043	1007.187	306.754	102368.7	4	20	10
2	1	0.042	1007.21	300.696	102529.9	4	20	10
2	1	0.044	1007.234	267.113	102672.8	5	21	10
2	1	0.054	1007.262	279.718	102822.5	5	21	10
2	1	0.052	1007.29	248.527	102955.6	6	22	10
2	1	0.05	1007.317	204.826	103063	6	22	10
2	1	0.074	1007.356	245.431	103194.6	7	23	10
2	1	0.091	1007.405	235.361	103320.7	7	23	10
2	1	0.137	1075.922	250.077	118444.5	8	0	11
2	1	0.11	1075.979	172.094	118532.9	8	0	11
2	1	6.489	1079.454	396.189	118745	9	1	11
2	1	33.155	1097.238	662.655	119100.5	9	1	11
2	1	35.352	1116.152	790.802	119523.6	10	2	11
2	1	24.695	1129.377	748.489	119924.4	10	2	11
2	1	15.37	1137.613	741.451	120321.7	11	3	11
2	1	14.145	1145.185	1048.056	120882.7	11	3	11
2	1	10.95	1150.93	1165.523	121494.3	12	4	11
2	1	7.333	1154.857	1032.432	122047.2	12	4	11
2	1	5.793	1157.894	1066.915	122606.5	13	5	11
2	1	5.216	1160.687	1172.906	123234.6	13	5	11
2	1	4.366	1163.023	1206.64	123880.2	14	6	11
2	1	3.388	1164.839	1161.132	124502.7	14	6	11
2	1	2.337	1166.09	1044.916	125062	15	7	11
2	1	1.478	1166.881	934.125	125562	16	8	11
2	1	0.995	1167.415	866.884	126026.5	16	8	11
2	1	0.782	1167.825	861.097	126478.1	17	9	11
2	1	0.574	1168.132	774.207	126892.5	17	9	11
2	1	0.483	1168.391	800.301	127320.9	18	10	11
2	1	0.339	1168.568	733.485	127705.4	18	10	11
2	1	0.224	1168.688	664.013	128061	19	11	11
2	1	0.151	1168.767	664.3	128409.8	19	11	11

2	1	0.056	1168.797	520	128688.1	20	12	11
2	1	-0.019	1168.787	281.945	128835.9	20	12	11
2	1	0.017	1168.797	436.525	129069.7	21	13	11
2	1	-0.002	1168.795	431.661	129301	21	13	11
2	1	-0.003	1168.793	478.191	129556.9	22	14	11
2	1	-0.011	1168.787	432.356	129783.4	22	14	11
2	1	-0.011	1168.781	369.659	129981.2	23	15	11
2	1	-0.013	1075.512	403.922	116003.9	0	16	11
2	1	-0.008	1075.508	384.265	116209.7	0	16	11
2	1	-0.001	1075.507	369.725	116407.6	1	17	11
2	1	0.006	1075.51	351.724	116596	1	17	11
2	1	0.016	1075.519	328.612	116772.3	2	18	11
2	1	0.023	1075.531	312.878	116939.7	2	18	11
2	1	0.025	1075.544	310.177	117105.7	3	19	11
2	1	0.033	1075.562	304.226	117268.6	3	19	11
2	1	0.042	1075.584	311.293	117435.3	4	20	11
2	1	0.053	1075.612	288.447	117589.9	4	20	11
2	1	0.059	1075.644	273.826	117736.5	5	21	11
2	1	0.068	1075.68	277.196	117882.1	5	21	11
2	1	0.087	1075.726	279.31	118031.7	6	22	11
2	1	0.105	1075.782	265.252	118173.7	7	23	11
2	1	0.126	1075.849	261.018	118310.5	7	23	11
2	1	0.089	1169.036	237.874	132419.5	8	0	12
2	1	-0.011	1168.776	325.98	130152.2	0	16	12
2	1	-0.01	1168.771	193.44	130255.7	0	16	12
2	1	-0.001	1168.77	340.117	130434.1	1	17	12
2	1	0.018	1168.779	345.766	130619.1	1	17	12
2	1	0.014	1168.787	342.959	130798.8	2	18	12
2	1	0.018	1168.796	316.239	130968.2	2	18	12
2	1	0.02	1168.807	328.143	131140.1	3	19	12
2	1	0.028	1168.821	318.464	131307.3	3	19	12
2	1	0.032	1168.838	311.281	131474	4	20	12
2	1	0.033	1168.856	297.73	131630.1	4	20	12
2	1	0.039	1168.877	298.25	131789.7	5	21	12
2	1	0.051	1168.903	299.513	131946.6	5	21	12
2	1	0.034	1168.921	186.94	132046.7	6	22	12
2	1	0.049	1168.947	202.871	132150.7	6	22	12
2	1	0.079	1168.989	268.87	132294.7	7	23	12
2	2	0.288	685.081	327.425	46648.36	8	0	8
2	2	0.45	685.322	560.956	46948.48	8	0	8
2	2	0.473	789.211	449.78	60442.27	8	0	8
2	2	11.288	795.253	764.361	60851.41	8	0	8
2	2	1.668	686.214	761.59	47355.93	9	1	8

2	2	8.788	690.928	726.245	47745.48	9	1	8
2	2	46.021	819.887	811.256	61285.66	9	1	8
2	2	21.782	702.587	918.338	48237.04	10	2	8
2	2	65.4	854.876	813.713	61721	10	2	8
2	2	65.984	890.177	803.21	62150.71	10	2	8
2	2	28.37	717.773	959.018	48750.38	11	3	8
2	2	29.615	733.626	1023.619	49298.3	11	3	8
2	2	65.78	925.461	820.984	62591.08	11	3	8
2	2	66.485	961.049	928.811	63088.25	11	3	8
2	2	25.819	747.453	1010.297	49839.38	12	4	8
2	2	19.467	757.879	1015.076	50383	12	4	8
2	2	65.418	996.048	983.645	63614.5	12	4	8
2	2	50.001	1022.798	1009.434	64154.55	12	4	8
2	2	14.16	765.459	1020.354	50929.18	13	5	8
2	2	10.918	771.303	1006.902	51468.15	13	5	8
2	2	36.849	1042.943	1048.819	64727.91	13	5	8
2	2	27.265	1057.545	1050.794	65290.66	13	5	8
2	2	8.041	775.609	905.895	51953.3	14	6	8
2	2	5.462	778.536	795.295	52379.45	14	6	8
2	2	19.644	1068.054	1035.464	65844.64	14	6	8
2	2	13.399	1075.222	997.333	66378.21	14	6	8
2	2	4.317	780.848	819.466	52818.32	15	7	8
2	2	3.146	782.531	826.239	53260.36	15	7	8
2	2	9.554	1080.339	990.498	66908.68	15	7	8
2	2	6.438	1083.787	917.277	67399.93	15	7	8
2	2	2.101	783.681	774.689	53684.29	16	8	8
2	2	1.498	784.483	753.53	54087.63	16	8	8
2	2	4.641	1086.271	897.484	67880.34	16	8	8
2	2	1.284	785.17	754.034	54491.25	17	9	8
2	2	1.095	785.756	704.607	54868.21	17	9	8
2	2	3.799	1088.304	876.08	68349.04	17	9	8
2	2	2.971	1089.895	768.389	68760.55	17	9	8
2	2	0.936	786.257	649.3	55215.59	18	10	8
2	2	2.429	1091.195	738.156	69155.47	18	10	8
2	2	1.831	1092.175	683.69	69521.24	18	10	8
2	2	0.79	786.68	605.216	55539.55	19	11	8
2	2	0.607	787.005	557.394	55837.75	19	11	8
2	2	1.384	1092.915	645.581	69866.8	19	11	8
2	2	0.833	1093.362	529.686	70150.63	19	11	8
2	2	0.476	787.26	548.362	56131.13	20	12	8
2	2	0.357	787.451	520.241	56409.6	20	12	8
2	2	0.275	1093.509	353.028	70339.69	20	12	8
2	2	0.317	1093.679	484.215	70599.01	20	12	8

2	2	0.236	787.577	476.442	56664.89	21	13	8
2	2	0.162	787.664	480.45	56922.34	21	13	8
2	2	0.185	1093.778	504.55	70868.95	21	13	8
2	2	0.074	1093.818	499.065	71135.95	21	13	8
2	2	0.105	787.72	436.409	57156.06	22	14	8
2	2	0.023	787.733	274.851	57303.41	22	14	8
2	2	-0.005	1093.815	487.46	71397.01	22	14	8
2	2	-0.018	1093.805	442.234	71633.73	22	14	8
2	2	0.053	787.761	393.504	57513.93	23	15	8
2	2	0.058	787.792	431.979	57745.04	23	15	8
2	2	-0.018	1093.796	428.933	71863.45	23	15	8
2	2	-0.017	1093.787	415.052	72085.5	23	15	8
2	2	0.036	787.811	316.663	57914.63	0	16	8
2	2	0.023	787.824	220.386	58032.6	0	16	8
2	2	0.051	787.851	325.112	58206.72	1	17	8
2	2	0.06	787.883	343.707	58390.6	2	18	8
2	2	0.077	787.924	343.592	58574.42	2	18	8
2	2	0.083	787.969	307.975	58739.19	3	19	8
2	2	0.118	788.032	354.809	58929.11	3	19	8
2	2	0.152	788.114	358.864	59121.3	4	20	8
2	2	0.178	788.209	330.852	59298.58	4	20	8
2	2	0.2	788.316	315.121	59467.35	5	21	8
2	2	0.223	788.436	313.74	59635.2	5	21	8
2	2	0.21	788.548	256.847	59772.68	6	22	8
2	2	0.221	788.666	241.061	59901.72	6	22	8
2	2	0.274	788.813	292.147	60058.1	7	23	8
2	2	0.27	788.958	267.554	60201.39	7	23	8
2	2	0.212	1094.892	236.093	75171	8	0	9
2	2	1.418	1095.635	344.743	75351.7	9	1	9
2	2	11.652	1101.879	479.007	75608.37	9	1	9
2	2	14.772	1109.786	479.337	75864.95	10	2	9
2	2	23.333	1122.276	838.593	76313.83	10	2	9
2	2	22.85	1134.507	1027.365	76863.76	11	3	9
2	2	17.915	1144.315	1081.094	77455.66	11	3	9
2	2	14.894	1152.283	1197.648	78096.4	12	4	9
2	2	11.519	1158.446	1228.514	78753.66	12	4	9
2	2	8.067	1162.856	1110.275	79360.61	13	5	9
2	2	5.893	1166.014	1110.221	79955.5	13	5	9
2	2	4.406	1168.376	1106.038	80548.46	14	6	9
2	2	2.932	1169.944	1047.58	81108.91	14	6	9
2	2	2.118	1171.078	1083.235	81689.05	15	7	9
2	2	1.448	1171.869	962.383	82214.62	16	8	9
2	2	1.2	1172.511	951.98	82723.93	16	8	9

2	2	1.094	1173.097	937.377	83225.95	17	9	9
2	2	1.004	1173.635	883.212	83698.95	17	9	9
2	2	0.928	1174.132	842.911	84150.15	18	10	9
2	2	0.808	1174.564	765.575	84559.73	18	10	9
2	2	0.687	1174.932	711.367	84940.31	19	11	9
2	2	0.552	1175.227	669.598	85298.55	19	11	9
2	2	0.34	1175.409	517.645	85575.77	20	12	9
2	2	0.205	1175.519	401.423	85790.65	20	12	9
2	2	0.195	1175.623	484.551	86049.88	21	13	9
2	2	0.156	1175.706	521.076	86328.66	21	13	9
2	2	0.118	1175.769	489.011	86590.41	22	14	9
2	2	0.1	1175.823	472.646	86843.41	23	15	9
2	2	0.088	1175.87	465.137	87092.52	23	15	9
2	2	-0.017	1093.777	417.445	72308.84	0	16	9
2	2	-0.016	1093.769	385.328	72514.98	1	17	9
2	2	-0.016	1093.76	384.416	72720.75	1	17	9
2	2	-0.015	1093.752	369.52	72918.45	2	18	9
2	2	-0.015	1093.744	380.799	73122.17	2	18	9
2	2	0.011	1093.75	403.457	73338.58	3	19	9
2	2	0.055	1093.78	367.43	73535.26	3	19	9
2	2	0.121	1093.844	382.226	73739.75	4	20	9
2	2	0.18	1093.941	378.111	73942.04	4	20	9
2	2	0.201	1094.048	347.999	74128.31	5	21	9
2	2	0.252	1094.183	368.161	74325.38	5	21	9
2	2	0.279	1094.332	362.931	74519.75	6	22	9
2	2	0.281	1094.483	346.782	74705.38	6	22	9
2	2	0.272	1094.629	319.091	74876.27	7	23	9
2	2	0.28	1094.779	314.317	75044.69	7	23	9
2	2	0.16	1176.99	200.735	89839.49	8	0	10
2	2	1.746	1177.924	618.415	90170.34	8	0	10
2	2	18.205	1187.669	801.065	90599.13	9	1	10
2	2	27.129	1202.206	867.792	91064.13	9	1	10
2	2	25.179	1215.677	953.153	91574.06	10	2	10
2	2	18.125	1225.378	996.206	92107.31	10	2	10
2	2	14.425	1233.099	1150.138	92722.95	11	3	10
2	2	11.822	1239.431	1215.553	93373.95	11	3	10
2	2	9.461	1244.497	1255.361	94046.27	12	4	10
2	2	7.291	1248.4	1202.329	94689.84	12	4	10
2	2	4.855	1251.001	1064.213	95260.09	13	5	10
2	2	3.083	1252.654	980.444	95785.71	13	5	10
2	2	1.983	1253.716	920.888	96278.64	14	6	10
2	2	1.259	1254.391	873.805	96747.09	15	7	10
2	2	0.84	1254.841	817.93	97185.14	15	7	10

2	2	0.614	1255.17	761.469	97592.95	16	8	10
2	2	0.757	1255.575	708.524	97972.41	16	8	10
2	2	0.616	1255.905	631.666	98310.88	17	9	10
2	2	0.351	1256.093	464.39	98559.58	17	9	10
2	2	0.386	1256.299	522.751	98839.25	18	10	10
2	2	0.313	1256.467	512.571	99114.05	18	10	10
2	2	0.267	1256.61	493.363	99378.27	19	11	10
2	2	0.14	1256.685	358.449	99570.14	19	11	10
2	2	0.087	1256.731	343.21	99753.95	20	12	10
2	2	0.057	1256.761	345.357	99938.8	20	12	10
2	2	0.039	1256.783	335.828	100118.7	21	13	10
2	2	0.028	1256.798	330.563	100295.6	22	14	10
2	2	0.009	1256.803	281.795	100446.5	22	14	10
2	2	0.006	1256.806	277.945	100595.4	23	15	10
2	2	0.007	1256.809	255.456	100732.1	23	15	10
2	2	0.071	1175.908	414.135	87314.2	0	16	10
2	2	0.067	1175.944	346.121	87499.66	0	16	10
2	2	0.077	1175.985	399.46	87713.82	1	17	10
2	2	0.079	1176.027	373.454	87913.72	1	17	10
2	2	0.083	1176.072	376.959	88115.6	2	18	10
2	2	0.092	1176.121	350.53	88303.23	2	18	10
2	2	0.101	1176.176	344.154	88487.55	3	19	10
2	2	0.128	1176.244	359.956	88680.42	3	19	10
2	2	0.143	1176.321	318.125	88851.06	4	20	10
2	2	0.163	1176.408	314.161	89019.14	4	20	10
2	2	0.17	1176.499	282.715	89170.47	5	21	10
2	2	0.198	1176.605	293.831	89327.83	5	21	10
2	2	0.184	1176.703	253.779	89463.67	6	22	10
2	2	0.174	1176.795	239.229	89589.2	7	23	10
2	2	0.205	1176.904	266.412	89732.1	7	23	10
2	2	0.111	1257.217	155.127	102164.7	8	0	11
2	2	0.409	1257.427	359.528	102349.5	8	0	11
2	2	7.242	1261.306	552.786	102645.5	9	1	11
2	2	16.416	1270.102	575.28	102953.8	9	1	11
2	2	14.693	1277.971	579.712	103264.3	10	2	11
2	2	8.607	1282.58	465.343	103513.5	10	2	11
2	2	4.299	1284.883	400.938	103728.2	11	3	11
2	2	4.613	1287.353	597.014	104047.9	11	3	11
2	2	3.672	1289.279	618.969	104372.6	12	4	11
2	2	2.775	1290.765	605.552	104696.9	12	4	11
2	2	3.07	1292.375	781.078	105106.5	13	5	11
2	2	2.625	1293.779	812.192	105541	14	6	11
2	2	1.821	1294.755	764.502	105950.4	14	6	11

2	2	1.186	1295.39	742.606	106348.4	15	7	11
2	2	1.067	1295.961	696.102	106721	15	7	11
2	2	0.661	1296.314	611.455	107048.1	16	8	11
2	2	0.48	1296.572	587.657	107363.3	16	8	11
2	2	0.383	1296.773	612.361	107684.1	17	9	11
2	2	0.302	1296.935	543.846	107975.4	17	9	11
2	2	0.234	1297.06	504.56	108245.5	18	10	11
2	2	0.17	1297.149	485.333	108499.7	18	10	11
2	2	0.123	1297.215	440.63	108735.9	19	11	11
2	2	0.082	1297.258	424.05	108958.3	19	11	11
2	2	0.021	1297.269	307.957	109123.3	20	12	11
2	2	-0.001	1297.268	178.623	109216.9	20	12	11
2	2	0.009	1297.273	288.145	109371.3	21	13	11
2	2	0.009	1297.278	316.878	109540.9	22	14	11
2	2	0.005	1297.281	306.058	109704.7	22	14	11
2	2	0	1297.281	274.727	109848.6	23	15	11
2	2	0.002	1297.282	263.911	109989.9	23	15	11
2	2	0.005	1256.812	230.063	100855.3	0	16	11
2	2	0.01	1256.817	228.373	100977.6	0	16	11
2	2	0.013	1256.824	215.791	101093.1	1	17	11
2	2	0.015	1256.832	204.21	101202.5	1	17	11
2	2	0.021	1256.844	189.304	101304	2	18	11
2	2	0.028	1256.859	191.502	101406.4	2	18	11
2	2	0.035	1256.878	184.248	101505.1	3	19	11
2	2	0.046	1256.903	180.532	101601.8	3	19	11
2	2	0.062	1256.936	170.12	101692.8	4	20	11
2	2	0.068	1256.972	157.972	101777.5	4	20	11
2	2	0.079	1257.015	150.087	101857.9	5	21	11
2	2	0.038	1257.034	64.724	101891.9	6	22	11
2	2	0.098	1257.087	156.978	101976	6	22	11
2	2	0.092	1257.136	134.321	102047.9	7	23	11
2	2	0.042	1257.158	64.288	102081.6	7	23	11
2	2	0.079	1297.567	155.76	111526.3	8	0	12
2	2	0.004	1297.284	202.219	110096	0	16	12
2	2	0.003	1297.286	159.362	110181.2	0	16	12
2	2	0.013	1297.292	218.477	110295.8	1	17	12
2	2	0.015	1297.3	214.363	110410.5	1	17	12
2	2	0.016	1297.309	213.311	110522.3	2	18	12
2	2	0.018	1297.318	209.994	110634.8	2	18	12
2	2	0.026	1297.332	212.414	110746.1	3	19	12
2	2	0.028	1297.347	197.491	110849.8	3	19	12
2	2	0.031	1297.363	182.553	110947.5	4	20	12
2	2	0.037	1297.383	197.446	111051	4	20	12

2	2	0.043	1297.406	172.367	111143.3	5	21	12
2	2	0.055	1297.434	179.836	111237.5	5	21	12
2	2	0.05	1297.461	133.569	111309	6	22	12
2	2	0.036	1297.479	74.575	111347.2	6	22	12
2	2	0.086	1297.526	181.77	111444.6	7	23	12
2	3	0.149	423.537	197.982	60941.57	8	0	8
2	3	0.241	423.666	342.448	61124.79	8	0	8
2	3	0.112	459.269	170.987	72725.45	8	0	8
2	3	0.232	459.393	302.023	72887.2	8	0	8
2	3	1.195	424.306	580.306	61435.57	9	1	8
2	3	4.991	462.065	611.688	73214.63	9	1	8
2	3	18.901	472.177	679.302	73578.05	9	1	8
2	3	4.661	426.804	533.453	61721.41	10	2	8
2	3	9.124	431.688	654.545	62071.78	10	2	8
2	3	26.002	486.088	723.19	73964.96	10	2	8
2	3	23.182	498.503	750.337	74366.8	10	2	8
2	3	11.523	437.859	818.272	62510.01	11	3	8
2	3	10.124	443.275	886.381	62984.22	11	3	8
2	3	16.942	507.586	763.678	74776.22	11	3	8
2	3	12.921	514.499	908.325	75262.17	11	3	8
2	3	8.702	447.938	941.57	63488.75	12	4	8
2	3	5.831	451.06	925.142	63983.95	12	4	8
2	3	9.542	519.604	950.087	75770.47	12	4	8
2	3	6.509	523.087	979.826	76294.68	12	4	8
2	3	3.889	453.142	922.148	64477.81	13	5	8
2	3	2.559	454.511	876.322	64946.64	13	5	8
2	3	4.219	525.396	985.645	76834.05	13	5	8
2	3	2.261	526.605	832.797	77279.59	13	5	8
2	3	1.629	455.384	755.109	65351.26	14	6	8
2	3	1.245	456.052	768.198	65763.09	14	6	8
2	3	0.625	526.94	573.855	77586.61	14	6	8
2	3	0.422	527.166	744.173	77984.95	14	6	8
2	3	1.066	456.622	856.43	66221.28	15	7	8
2	3	0.792	457.046	830.165	66665.42	15	7	8
2	3	0.282	527.317	798.157	78412.41	15	7	8
2	3	0.594	457.371	775.57	67090.05	16	8	8
2	3	0.164	527.404	769.25	78824.17	16	8	8
2	3	0.082	527.448	705.75	79201.95	16	8	8
2	3	0.516	457.647	779.013	67507.03	17	9	8
2	3	0.496	457.913	783.9	67926.41	17	9	8
2	3	0.085	527.494	697.229	79574.96	17	9	8
2	3	0.105	527.55	692.644	79945.91	17	9	8
2	3	0.459	458.159	722.539	68312.97	18	10	8

2	3	0.419	458.383	683.154	68678.65	18	10	8
2	3	0.078	527.592	649.745	80293.53	18	10	8
2	3	0.053	527.621	663.356	80648.43	18	10	8
2	3	0.304	458.546	605.782	69002.74	19	11	8
2	3	0.209	458.658	559.964	69302.32	19	11	8
2	3	-0.005	527.618	573.674	80955.66	19	11	8
2	3	-0.004	527.615	450.699	81197.29	19	11	8
2	3	0.134	458.729	532.09	69586.99	20	12	8
2	3	0.058	458.76	424.418	69814.29	20	12	8
2	3	-0.003	527.614	311.451	81363.91	20	12	8
2	3	-0.003	527.612	348.943	81550.79	20	12	8
2	3	0.023	458.773	425.988	70042.66	21	13	8
2	3	0.006	458.776	399.332	70256.53	21	13	8
2	3	-0.004	527.61	369.9	81748.69	21	13	8
2	3	-0.004	527.608	378.915	81951.52	21	13	8
2	3	-0.001	458.775	309.764	70422.43	22	14	8
2	3	-0.002	458.774	222.053	70541.41	22	14	8
2	3	-0.004	527.606	373.132	82151.35	22	14	8
2	3	0.001	458.775	339.639	70723.12	23	15	8
2	3	0.005	458.778	347.293	70909.02	23	15	8
2	3	-0.003	527.604	332.558	82329.36	23	15	8
2	3	-0.003	527.603	319.851	82500.57	23	15	8
2	3	0.003	458.779	281.906	71059.99	0	16	8
2	3	0.004	458.781	203.209	71168.77	1	17	8
2	3	0.013	458.789	265.605	71310.94	1	17	8
2	3	0.015	458.797	221.242	71429.3	2	18	8
2	3	0.029	458.812	249.535	71562.8	2	18	8
2	3	0.036	458.831	243.837	71693.33	3	19	8
2	3	0.045	458.856	243.204	71823.45	3	19	8
2	3	0.057	458.886	237.982	71950.96	4	20	8
2	3	0.072	458.925	238.25	72078.69	4	20	8
2	3	0.089	458.973	222.742	72197.85	5	21	8
2	3	0.12	459.037	249.127	72331.13	5	21	8
2	3	0.099	459.09	183.639	72429.48	6	22	8
2	3	0.104	459.145	176.94	72524.2	6	22	8
2	3	0.119	459.209	204.931	72633.84	7	23	8
2	3	0.076	527.754	173.369	84594.88	8	0	9
2	3	0.042	527.776	94.645	84645.55	8	0	9
2	3	0.794	528.193	506.535	84911.2	9	1	9
2	3	5.022	530.881	657.156	85262.95	9	1	9
2	3	5.355	533.747	548.003	85556.29	10	2	9
2	3	2.17	534.91	254.839	85692.77	10	2	9
2	3	3.488	536.778	480.414	85950.06	11	3	9

2	3	3.336	538.602	610.468	86283.95	11	3	9
2	3	2.867	540.136	703.662	86660.41	12	4	9
2	3	2.478	541.462	792.455	87084.38	12	4	9
2	3	2.051	542.584	770.046	87505.76	13	5	9
2	3	1.829	543.563	769.346	87917.79	14	6	9
2	3	1.83	544.544	813.581	88353.73	14	6	9
2	3	1.749	545.48	840.191	88803.23	15	7	9
2	3	1.55	546.31	800.789	89232.1	15	7	9
2	3	1.328	547.035	732.516	89632.13	16	8	9
2	3	1.107	547.627	705.386	90009.71	16	8	9
2	3	0.965	548.144	696.705	90382.84	17	9	9
2	3	0.821	548.584	643.179	90727.12	17	9	9
2	3	0.759	548.99	633.62	91066.28	18	10	9
2	3	0.665	549.346	599.424	91386.98	18	10	9
2	3	0.586	549.659	590.046	91702.65	19	11	9
2	3	0.49	549.921	549.765	91996.92	19	11	9
2	3	0.35	550.109	463.867	92245.35	20	12	9
2	3	0.232	550.233	362.851	92439.48	20	12	9
2	3	0.219	550.35	408.586	92658.07	21	13	9
2	3	0.186	550.45	421.164	92883.39	22	14	9
2	3	0.151	550.531	429.347	93113.21	22	14	9
2	3	0.117	550.593	396.216	93325.41	23	15	9
2	3	0.091	550.642	347.191	93511.25	23	15	9
2	3	-0.004	527.601	359.157	82692.72	0	16	9
2	3	-0.003	527.599	281.567	82843.36	0	16	9
2	3	-0.003	527.598	290.611	82998.91	1	17	9
2	3	-0.003	527.596	286.272	83152.07	1	17	9
2	3	-0.003	527.595	268.619	83295.78	2	18	9
2	3	-0.002	527.593	251.51	83430.48	2	18	9
2	3	-0.003	527.592	294.382	83588.3	3	19	9
2	3	-0.002	527.591	239.487	83716.42	3	19	9
2	3	-0.002	527.589	231.377	83840.21	4	20	9
2	3	0.001	527.59	218.354	83957.03	4	20	9
2	3	0.014	527.598	218.533	84074.07	5	21	9
2	3	0.033	527.616	210.009	84186.48	5	21	9
2	3	0.058	527.647	234.343	84311.98	6	22	9
2	3	0.057	527.677	180.98	84408.86	7	23	9
2	3	0.069	527.714	173.977	84502.03	7	23	9
2	3	0.101	551.383	162.969	95819.68	8	0	10
2	3	0.741	551.779	454.56	96062.87	8	0	10
2	3	10.691	557.505	730.773	96454.23	9	1	10
2	3	14.247	565.135	752.425	96857.2	9	1	10
2	3	10.885	570.959	793.288	97281.61	10	2	10

2	3	7.047	574.732	755.939	97686.45	10	2	10
2	3	5.101	577.463	835.461	98133.66	11	3	10
2	3	4.227	579.727	876.182	98602.9	11	3	10
2	3	3.523	581.613	846.122	99055.81	12	4	10
2	3	3.909	583.706	934.458	99556.27	13	5	10
2	3	3.571	585.621	979.989	100081.6	13	5	10
2	3	3.037	587.248	992.838	100613.6	14	6	10
2	3	2.42	588.544	956.408	101125.6	14	6	10
2	3	1.841	589.53	897.572	101606.5	15	7	10
2	3	1.36	590.259	839.208	102056.4	15	7	10
2	3	0.988	590.788	794.3	102481.4	16	8	10
2	3	0.801	591.217	691.467	102851.9	16	8	10
2	3	0.509	591.49	529.329	103135.4	17	9	10
2	3	0.641	591.833	663.381	103490.7	17	9	10
2	3	0.537	592.12	627.69	103826.8	18	10	10
2	3	0.414	592.342	602.507	104149.7	18	10	10
2	3	0.303	592.505	550.885	104444.5	19	11	10
2	3	0.156	592.588	397.07	104657.2	19	11	10
2	3	0.093	592.638	378.195	104859.8	20	12	10
2	3	0.085	592.683	466.838	105109.5	21	13	10
2	3	0.053	592.712	431.283	105340.5	21	13	10
2	3	0.038	592.732	392.172	105550.5	22	14	10
2	3	0.028	592.746	378.502	105753.2	22	14	10
2	3	0.019	592.757	357.721	105944.7	23	15	10
2	3	0.015	592.765	332.815	106122.9	23	15	10
2	3	0.084	550.687	348.644	93697.88	0	16	10
2	3	0.068	550.724	316.935	93867.88	0	16	10
2	3	0.077	550.765	354.611	94057.89	1	17	10
2	3	0.075	550.805	327.905	94233.41	1	17	10
2	3	0.068	550.841	318.021	94403.73	2	18	10
2	3	0.068	550.878	310.695	94570.04	2	18	10
2	3	0.074	550.917	301.525	94731.44	3	19	10
2	3	0.073	550.957	273.689	94878.24	3	19	10
2	3	0.093	551.006	307.012	95042.75	4	20	10
2	3	0.095	551.057	266.126	95185.2	5	21	10
2	3	0.111	551.116	274.947	95332.3	5	21	10
2	3	0.108	551.174	216.101	95448.03	6	22	10
2	3	0.074	551.214	141.55	95523.84	6	22	10
2	3	0.061	551.246	105.765	95579.34	7	23	10
2	3	0.155	551.329	285.684	95732.49	7	23	10
2	3	0.123	593.711	126.278	108405.3	8	0	11
2	3	0.732	594.096	409.149	108620.1	8	0	11
2	3	12.456	600.77	618.507	108951.5	9	1	11

2	3	23.793	613.512	646.29	109297.6	9	1	11
2	3	20.604	624.547	668.537	109655.6	10	2	11
2	3	12.436	631.214	537.202	109943.6	10	2	11
2	3	3.358	633.01	204.788	110053.2	11	3	11
2	3	0.828	633.454	104.675	110109.3	12	4	11
2	3	1.075	634.018	83.617	110153.1	12	4	11
2	3	0.885	634.492	136.678	110226.3	13	5	11
2	3	0.297	634.648	66.118	110261	13	5	11
2	3	1.405	635.399	246.058	110392.6	14	6	11
2	3	1.199	636.042	248.846	110526	14	6	11
2	3	0.933	636.541	256.458	110663.3	15	7	11
2	3	0.669	636.899	247.693	110795.9	15	7	11
2	3	0.36	637.092	197.292	110901.5	16	8	11
2	3	0.166	637.181	145.387	110979.5	16	8	11
2	3	0.225	637.299	103.265	111033.6	17	9	11
2	3	0.144	637.376	179.575	111129.8	17	9	11
2	3	0.044	637.399	151.764	111210.9	18	10	11
2	3	0.135	637.47	118.771	111273.2	18	10	11
2	3	0.036	637.489	154.763	111356.2	19	11	11
2	3	0.094	637.539	136.952	111428	19	11	11
2	3	0.005	637.541	148.039	111507.3	20	12	11
2	3	0.057	637.571	125.247	111572.9	21	13	11
2	3	0.004	637.573	176.377	111667.4	21	13	11
2	3	0.01	637.578	156.373	111751.1	22	14	11
2	3	-0.001	637.578	122.189	111816.5	22	14	11
2	3	0.007	637.582	112.745	111875.6	23	15	11
2	3	0.002	637.583	144.871	111953.1	23	15	11
2	3	0.027	592.779	364.905	106318.3	0	16	11
2	3	0.026	592.793	328.632	106494.2	0	16	11
2	3	0.033	592.811	336.337	106674.2	1	17	11
2	3	0.039	592.832	309.692	106840.3	1	17	11
2	3	0.054	592.861	302.476	107002.3	2	18	11
2	3	0.067	592.896	270.316	107146.9	2	18	11
2	3	0.094	592.947	299.698	107307.5	3	19	11
2	3	0.112	593.007	263.143	107448.4	4	20	11
2	3	0.159	593.092	301.422	107609.8	4	20	11
2	3	0.164	593.18	270.269	107754.6	5	21	11
2	3	0.159	593.263	223.339	107871.8	5	21	11
2	3	0.17	593.355	227.952	107994	6	22	11
2	3	0.175	593.448	221.053	108112.4	6	22	11
2	3	0.186	593.546	217.328	108226.2	7	23	11
2	3	0.188	593.646	210.657	108339	7	23	11
2	3	0.091	638.037	88.66	112848	8	0	12

2	3	0.008	637.587	60.548	111984.9	0	16	12
2	3	0.01	637.593	130.716	112054.8	0	16	12
2	3	0.023	637.605	134.729	112125.5	1	17	12
2	3	0.016	637.613	124.549	112192.1	1	17	12
2	3	0.022	637.625	98.445	112243.8	2	18	12
2	3	0.025	637.638	119.895	112307.9	2	18	12
2	3	0.026	637.652	67.455	112343.3	3	19	12
2	3	0.06	637.683	125.984	112409.4	3	19	12
2	3	0.071	637.721	138.87	112483.8	4	20	12
2	3	0.065	637.755	86.654	112529.2	5	21	12
2	3	0.088	637.802	124.288	112595.7	5	21	12
2	3	0.067	637.837	67.387	112631	6	22	12
2	3	0.1	637.889	118.179	112692.9	6	22	12
2	3	0.084	637.934	86.255	112738.1	7	23	12
2	3	0.106	637.99	120.253	112802.5	7	23	12
2	4	0.037	43.298	27.001	15738.91	8	0	8
2	4	0.031	43.314	24.193	15751.86	8	0	8
2	4	0.04	44.149	24.885	16347.27	8	0	8
2	4	0.043	44.172	18.758	16357.31	8	0	8
2	4	0.03	43.33	21.674	15763.45	9	1	8
2	4	0.064	43.364	22.402	15775.46	9	1	8
2	4	0.177	44.267	18.871	16367.42	9	1	8
2	4	-0.196	44.162	19.243	16377.71	9	1	8
2	4	0.118	43.427	31.71	15792.45	10	2	8
2	4	-0.104	43.372	38.022	15812.79	10	2	8
2	4	0.002	44.163	20.892	16388.89	10	2	8
2	4	-0.001	44.163	62.444	16422.37	10	2	8
2	4	-0.041	43.35	32.878	15830.39	11	3	8
2	4	0	43.35	38.62	15851.06	11	3	8
2	4	0	44.162	26.55	16436.58	11	3	8
2	4	0.033	44.18	63.935	16470.79	11	3	8
2	4	0.004	43.352	46.599	15876.04	12	4	8
2	4	0.024	43.364	31.418	15892.85	12	4	8
2	4	-0.024	44.167	25.863	16484.63	12	4	8
2	4	0.057	44.198	62.141	16517.89	12	4	8
2	4	0.096	43.416	37.985	15913.19	13	5	8
2	4	-0.045	43.392	28.714	15928.55	13	5	8
2	4	-0.001	44.198	60.019	16550.71	13	5	8
2	4	0.124	43.458	39.676	15949.82	14	6	8
2	4	0.143	43.535	42.091	15972.38	14	6	8
2	4	0	44.197	27.314	16565.33	14	6	8
2	4	0	44.197	17.552	16574.72	14	6	8
2	4	0.183	43.633	51.329	15999.84	15	7	8

2	4	0	44.197	17.553	16584.11	15	7	8
2	4	0	44.197	17.459	16593.47	15	7	8
2	4	0.188	43.734	52.282	16027.82	16	8	8
2	4	-0.001	43.733	53.868	16057.32	16	8	8
2	4	0	44.197	26.824	16607.83	16	8	8
2	4	0	44.197	19.17	16618.08	16	8	8
2	4	0.001	43.734	15.288	16065.5	17	9	8
2	4	0.005	43.737	15.371	16073.72	17	9	8
2	4	0	44.197	19.04	16628.27	17	9	8
2	4	0	44.197	19.64	16638.79	17	9	8
2	4	0.018	43.746	15.091	16081.79	18	10	8
2	4	0.02	43.757	16.021	16090.37	18	10	8
2	4	0	44.197	27.915	16653.72	18	10	8
2	4	0	44.196	20.097	16664.48	18	10	8
2	4	0.023	43.769	15.773	16098.81	19	11	8
2	4	0.022	43.781	14.774	16106.71	19	11	8
2	4	0	44.196	21.178	16675.82	19	11	8
2	4	0	44.196	20.462	16686.78	19	11	8
2	4	0.025	43.794	19.876	16117.35	20	12	8
2	4	0.029	43.81	16.855	16126.37	20	12	8
2	4	0	44.196	19.915	16697.44	20	12	8
2	4	0	44.196	17.888	16707.02	20	12	8
2	4	0.023	43.823	16.221	16135.07	21	13	8
2	4	0.024	43.836	17.333	16144.35	21	13	8
2	4	0	44.196	22.496	16719.06	21	13	8
2	4	0.024	43.849	18.693	16154.36	22	14	8
2	4	0.001	44.197	18.265	16728.84	22	14	8
2	4	0.001	44.197	19.469	16739.26	22	14	8
2	4	0.027	43.863	14.055	16161.89	23	15	8
2	4	0.026	43.877	15.548	16170.22	23	15	8
2	4	0.002	44.198	23.167	16751.66	23	15	8
2	4	0.003	44.2	21.849	16763.35	23	15	8
2	4	0.032	43.895	18.407	16180.07	0	16	8
2	4	0.03	43.911	18.54	16189.99	0	16	8
2	4	0.032	43.928	15.594	16198.34	1	17	8
2	4	0.03	43.944	19.457	16208.75	1	17	8
2	4	0.034	43.962	18.831	16218.83	2	18	8
2	4	0.033	43.98	19.583	16229.31	2	18	8
2	4	0.032	43.997	20.944	16240.52	3	19	8
2	4	0.031	44.013	19.944	16251.19	3	19	8
2	4	0.028	44.029	23.751	16263.92	4	20	8
2	4	0.032	44.046	20.276	16274.78	4	20	8
2	4	0.033	44.063	22.935	16287.05	5	21	8

2	4	0.027	44.078	24.023	16299.91	5	21	8
2	4	0.031	44.094	21.169	16311.25	6	22	8
2	4	0.034	44.112	21.083	16322.53	7	23	8
2	4	0.028	44.127	21.312	16333.93	7	23	8
2	4	0.033	44.356	21.918	16951.67	8	0	9
2	4	0.034	44.374	14.962	16959.51	8	0	9
2	4	0.063	44.408	19.04	16969.71	9	1	9
2	4	0.07	44.445	17.986	16979.34	9	1	9
2	4	0.023	44.458	36.692	16998.97	10	2	9
2	4	0.121	44.523	77.029	17040.23	10	2	9
2	4	0.103	44.578	81.259	17083.77	11	3	9
2	4	-0.001	44.577	102.35	17139.72	11	3	9
2	4	0.019	44.587	57.193	17170.32	12	4	9
2	4	0.145	44.665	64.775	17204.99	13	5	9
2	4	-0.001	44.664	100.754	17260.1	13	5	9
2	4	0	44.665	24.481	17273.21	14	6	9
2	4	0.017	44.674	23.605	17285.86	14	6	9
2	4	0.052	44.702	23.956	17298.68	15	7	9
2	4	0.264	44.843	119.229	17362.5	15	7	9
2	4	0	44.843	72.356	17402.04	16	8	9
2	4	0.045	44.867	34.264	17420.37	16	8	9
2	4	0.034	44.885	22.186	17432.26	17	9	9
2	4	0.043	44.908	20.785	17443.38	17	9	9
2	4	0.04	44.93	19.335	17453.73	18	10	9
2	4	0.044	44.953	19.166	17463.98	18	10	9
2	4	0.04	44.974	18.251	17473.75	19	11	9
2	4	0.051	45.001	20.154	17484.54	20	12	9
2	4	0.041	45.023	18.915	17494.67	20	12	9
2	4	0.046	45.048	17.782	17504.18	21	13	9
2	4	0.043	45.071	18.439	17514.04	21	13	9
2	4	0.048	45.096	18.675	17524.04	22	14	9
2	4	0.046	45.121	18.468	17533.92	22	14	9
2	4	0.046	45.145	18.491	17543.83	23	15	9
2	4	0.046	45.17	19.058	17554.03	23	15	9
2	4	0.004	44.202	19.854	16773.97	0	16	9
2	4	0.006	44.206	20.651	16785.02	0	16	9
2	4	0.008	44.21	21.801	16796.69	1	17	9
2	4	0.008	44.214	19.844	16807.31	1	17	9
2	4	0.01	44.219	22.153	16819.16	2	18	9
2	4	0.013	44.226	21.812	16830.86	2	18	9
2	4	0.013	44.233	21.908	16842.59	3	19	9
2	4	0.017	44.242	23.083	16854.94	3	19	9
2	4	0.02	44.253	22.672	16867.07	4	20	9

2	4	0.022	44.265	24.701	16880.29	5	21	9
2	4	0.025	44.278	21.925	16892.03	5	21	9
2	4	0.026	44.293	23.761	16904.75	6	22	9
2	4	0.026	44.307	20.983	16915.98	6	22	9
2	4	0.03	44.323	23.887	16928.77	7	23	9
2	4	0.03	44.339	20.842	16939.94	7	23	9
2	4	0.039	45.47	27.64	17749.21	8	0	10
2	4	0.087	45.517	20.367	17760.11	8	0	10
2	4	0.026	45.531	33.074	17777.83	9	1	10
2	4	-0.047	45.506	60.674	17810.29	9	1	10
2	4	-0.045	45.482	39.909	17831.66	10	2	10
2	4	0.003	45.484	44.884	17855.7	10	2	10
2	4	0.12	45.548	45.662	17880.14	11	3	10
2	4	0.171	45.639	43.037	17903.19	12	4	10
2	4	0.119	45.703	35.126	17921.99	12	4	10
2	4	0.288	45.857	45.816	17946.53	13	5	10
2	4	0.218	45.974	41.454	17968.74	13	5	10
2	4	0.208	46.085	39.673	17990	14	6	10
2	4	0.239	46.213	44.641	18013.9	14	6	10
2	4	0.211	46.326	38.038	18034.28	15	7	10
2	4	0.183	46.425	33.079	18052.01	15	7	10
2	4	0.17	46.516	30.657	18068.43	16	8	10
2	4	0.112	46.576	30.831	18084.94	16	8	10
2	4	0.139	46.651	29.429	18100.69	17	9	10
2	4	0.138	46.725	27.037	18115.17	17	9	10
2	4	0.135	46.797	26.047	18129.14	18	10	10
2	4	0.129	46.866	24.756	18142.39	19	11	10
2	4	0.124	46.933	24.195	18155.34	19	11	10
2	4	0.113	46.994	23.792	18168.08	20	12	10
2	4	0.114	47.055	21.858	18179.79	20	12	10
2	4	0.107	47.112	21.648	18191.38	21	13	10
2	4	0.104	47.168	21.659	18202.97	21	13	10
2	4	0.107	47.225	21.571	18214.52	22	14	10
2	4	0.098	47.278	21.502	18226.04	22	14	10
2	4	0.1	47.332	23.993	18238.89	23	15	10
2	4	0.091	47.38	20.798	18250.01	23	15	10
2	4	0.043	45.193	21.108	17565.32	0	16	10
2	4	0.039	45.214	19.458	17575.77	0	16	10
2	4	0.048	45.239	20.298	17586.64	1	17	10
2	4	0.037	45.259	24.206	17599.6	1	17	10
2	4	0.037	45.279	25.512	17613.26	2	18	10
2	4	0.032	45.297	22.504	17625.31	2	18	10
2	4	0.037	45.316	26.133	17639.3	3	19	10

2	4	0.033	45.334	22.638	17651.43	4	20	10
2	4	0.032	45.351	22.419	17663.45	4	20	10
2	4	0.033	45.369	25.965	17677.35	5	21	10
2	4	0.035	45.387	24.255	17690.32	5	21	10
2	4	0.03	45.404	23.243	17702.77	6	22	10
2	4	0.025	45.417	12.688	17709.42	6	22	10
2	4	0.034	45.435	23.507	17722.03	7	23	10
2	4	0.028	45.45	23.131	17734.42	7	23	10
2	4	0.066	48.075	12.247	18427.2	8	0	11
2	4	0.123	48.141	18.898	18437.32	8	0	11
2	4	0.185	48.24	17.494	18446.71	9	1	11
2	4	-0.296	48.081	17.707	18456.18	9	1	11
2	4	0.001	48.082	15.817	18464.65	10	2	11
2	4	0.239	48.21	59.931	18496.78	11	3	11
2	4	0.236	48.336	41.696	18519.09	11	3	11
2	4	0.1	48.389	22.428	18530.86	12	4	11
2	4	0.223	48.508	22.164	18542.73	12	4	11
2	4	0.105	48.563	19.626	18553.01	13	5	11
2	4	0.209	48.675	31.541	18569.91	13	5	11
2	4	0.266	48.817	26.101	18583.87	14	6	11
2	4	0.222	48.936	28.423	18599.11	14	6	11
2	4	0.2	49.043	24.041	18611.98	15	7	11
2	4	0.174	49.136	27.601	18626.75	15	7	11
2	4	0.151	49.217	22.461	18638.78	16	8	11
2	4	0.144	49.293	14.832	18646.57	16	8	11
2	4	0.168	49.383	22.917	18658.83	17	9	11
2	4	0.151	49.464	20.565	18669.84	17	9	11
2	4	0.139	49.537	13.221	18676.77	18	10	11
2	4	0.135	49.61	19.396	18687.15	19	11	11
2	4	0.127	49.676	12.431	18693.68	19	11	11
2	4	0.125	49.743	18.411	18703.54	20	12	11
2	4	0.117	49.804	11.029	18709.32	20	12	11
2	4	0.121	49.869	17.688	18718.79	21	13	11
2	4	0.113	49.929	17.153	18727.98	21	13	11
2	4	0.112	49.989	16.814	18736.98	22	14	11
2	4	0.107	50.045	9.765	18742.1	22	14	11
2	4	0.102	50.1	16.561	18750.96	23	15	11
2	4	0.12	50.163	9.541	18755.96	23	15	11
2	4	0.095	47.431	21.491	18261.52	0	16	11
2	4	0.089	47.478	23.759	18274.25	0	16	11
2	4	0.09	47.527	21.373	18285.69	1	17	11
2	4	0.09	47.575	22.711	18297.88	1	17	11
2	4	0.086	47.621	23.337	18310.36	2	18	11

2	4	0.085	47.666	22.381	18322.33	3	19	11
2	4	0.085	47.712	20.72	18333.43	3	19	11
2	4	0.078	47.754	20.76	18344.55	4	20	11
2	4	0.085	47.799	21.946	18356.31	4	20	11
2	4	0.078	47.841	20.672	18367.38	5	21	11
2	4	0.076	47.881	18.57	18377.13	5	21	11
2	4	0.08	47.924	23.701	18389.83	6	22	11
2	4	0.074	47.963	18.617	18399.79	6	22	11
2	4	0.079	48.005	21.303	18410.95	7	23	11
2	4	0.068	48.041	18.584	18420.92	7	23	11
2	4	0.079	50.892	15.952	18854.36	8	0	12
2	4	0.093	50.212	16.979	18765.05	0	16	12
2	4	0.105	50.267	8.008	18769.25	0	16	12
2	4	0.095	50.318	16.843	18778.26	1	17	12
2	4	0.085	50.363	9.168	18783.07	1	17	12
2	4	0.105	50.419	14.611	18790.89	2	18	12
2	4	0.088	50.465	7.698	18794.93	2	18	12
2	4	0.09	50.512	7.151	18798.68	3	19	12
2	4	0.091	50.561	15.646	18807.06	4	20	12
2	4	0.083	50.605	6.632	18810.54	4	20	12
2	4	0.083	50.649	15.338	18818.74	5	21	12
2	4	0.076	50.689	8.098	18822.99	5	21	12
2	4	0.08	50.732	13.84	18830.39	6	22	12
2	4	0.078	50.772	7.333	18834.15	6	22	12
2	4	0.077	50.813	15.065	18842.22	7	23	12
2	4	0.073	50.851	7.216	18846.01	7	23	12
3	1	0.336	107.397	369.501	60167.63	8	0	8
3	1	0.252	107.534	299.256	60331.14	8	0	8
3	1	0.056	119.724	233.621	71599.31	8	0	8
3	1	0.167	119.816	328.989	71779.25	8	0	8
3	1	0.132	107.606	94.81	60382.95	9	1	8
3	1	0.432	107.842	129.951	60453.98	9	1	8
3	1	1.69	120.74	500.932	72053.09	9	1	8
3	1	3.95	122.899	594.445	72378.05	9	1	8
3	1	0.803	108.272	170.035	60545.05	10	2	8
3	1	5.056	125.662	768.493	72797.95	10	2	8
3	1	3.786	127.731	783.05	73226.02	10	2	8
3	1	4.271	110.605	960.507	61069.59	11	3	8
3	1	3.529	112.532	968.129	61598.3	11	3	8
3	1	2.441	129.066	728.362	73624.4	11	3	8
3	1	2.307	113.793	830.841	62052.49	12	4	8
3	1	1.653	114.696	741.795	62457.59	12	4	8
3	1	1.887	130.098	762.1	74041.02	12	4	8

3	1	1.47	130.902	772.536	74463.55	12	4	8
3	1	1.211	115.358	643.275	62809.43	13	5	8
3	1	1.202	116.015	736.677	63212.14	13	5	8
3	1	1.213	131.565	797.764	74899.66	13	5	8
3	1	0.808	132.006	724.487	75295.91	13	5	8
3	1	0.911	116.512	738.078	63615.21	14	6	8
3	1	0.742	116.918	728.902	64013.28	14	6	8
3	1	0.432	132.243	560.182	75602.15	14	6	8
3	1	0.488	132.509	634.647	75949.27	14	6	8
3	1	0.655	117.268	761.291	64421.2	15	7	8
3	1	0.533	117.554	722.463	64808.12	15	7	8
3	1	0.454	132.757	658.574	76309.47	15	7	8
3	1	0.381	132.966	640.026	76659.7	15	7	8
3	1	0.406	117.776	641.854	65159	16	8	8
3	1	0.321	133.141	626.676	77001.94	16	8	8
3	1	0.235	133.269	597.938	77328.48	16	8	8
3	1	0.307	117.941	612.443	65487.34	17	9	8
3	1	0.27	118.085	636.797	65828.02	17	9	8
3	1	0.199	133.378	567.476	77638.85	17	9	8
3	1	0.21	118.197	611.947	66155.76	18	10	8
3	1	0.169	118.288	581.865	66467.05	18	10	8
3	1	0.16	133.466	531.061	77929.46	18	10	8
3	1	0.138	133.541	522.861	78215	18	10	8
3	1	0.15	118.37	557.097	66771.76	19	11	8
3	1	0.15	118.451	567.925	67076.23	19	11	8
3	1	0.134	133.615	521.614	78500.44	19	11	8
3	1	0.035	133.634	395.47	78716.52	19	11	8
3	1	0.076	118.491	448.38	67316.48	20	12	8
3	1	0.059	118.523	379.606	67519.99	20	12	8
3	1	0	133.634	297.742	78879.2	20	12	8
3	1	0.063	133.667	489.99	79141.88	20	12	8
3	1	0.103	118.578	502.727	67789.23	21	13	8
3	1	0.115	118.641	492.485	68058.59	21	13	8
3	1	0.056	133.697	495.404	79406.92	21	13	8
3	1	0.047	133.723	473.664	79660.59	21	13	8
3	1	0.129	118.711	526.597	68346.61	22	14	8
3	1	0.151	118.794	560.534	68653.19	22	14	8
3	1	0.053	133.751	478.572	79916.9	22	14	8
3	1	0.051	133.779	466.73	80166.86	22	14	8
3	1	0.157	118.88	581.995	68971.18	23	15	8
3	1	0.055	133.808	482.907	80425.22	23	15	8
3	1	0.062	133.841	517.693	80696.72	23	15	8
3	1	0.132	118.952	534.957	69263.33	0	16	8

3	1	0.107	119.011	441.314	69504.34	0	16	8
3	1	0.121	119.075	456.496	69748.81	1	17	8
3	1	0.148	119.154	405.976	69966.01	1	17	8
3	1	0.124	119.222	303.395	70131.87	2	18	8
3	1	0.117	119.286	273.017	70281.04	2	18	8
3	1	0.096	119.339	259.568	70422.79	3	19	8
3	1	0.098	119.392	261.814	70565.91	3	19	8
3	1	0.101	119.448	268.702	70713.03	4	20	8
3	1	0.09	119.497	263.667	70857.24	4	20	8
3	1	0.084	119.543	252.859	70995.61	5	21	8
3	1	0.069	119.581	218.066	71115.06	6	22	8
3	1	0.058	119.612	190.515	71216.98	6	22	8
3	1	0.076	119.654	238.498	71347.36	7	23	8
3	1	0.073	119.694	227.163	71471.6	7	23	8
3	1	0.095	134.237	226.819	83627.27	8	0	9
3	1	0.514	134.513	550.639	83922.48	8	0	9
3	1	2.344	135.767	571.394	84228.17	9	1	9
3	1	2.754	137.273	413.633	84454.41	10	2	9
3	1	3.511	139.193	503.35	84729.57	10	2	9
3	1	3.27	140.979	608.961	85062.13	11	3	9
3	1	2.848	142.534	681.392	85434.25	11	3	9
3	1	2.199	143.736	670.715	85800.91	12	4	9
3	1	1.564	144.59	706.987	86187.2	12	4	9
3	1	1.267	145.283	770.613	86608.46	13	5	9
3	1	0.913	145.782	761.978	87025.22	13	5	9
3	1	0.465	146.036	613.633	87360.5	14	6	9
3	1	0.039	146.057	384.245	87570.34	14	6	9
3	1	0.223	146.179	562.083	87877.46	15	7	9
3	1	0.201	146.289	581.589	88195.4	16	8	9
3	1	0.005	146.291	413.072	88421.55	16	8	9
3	1	-0.004	146.289	320.846	88596.77	17	9	9
3	1	0.062	146.323	475.834	88857.03	17	9	9
3	1	0.24	146.455	600.332	89185.21	18	10	9
3	1	0.263	146.598	612.263	89519.91	18	10	9
3	1	0.238	146.729	607.818	89852.02	19	11	9
3	1	0.221	146.85	597.26	90178.52	19	11	9
3	1	0.183	146.947	593.566	90496.41	20	12	9
3	1	0.1	147.002	510.429	90775.16	20	12	9
3	1	0.109	147.061	560.983	91075.59	21	13	9
3	1	0.091	147.111	558.538	91380.62	22	14	9
3	1	-0.002	147.109	340.738	91563.1	22	14	9
3	1	0.028	147.124	494.535	91822.46	23	15	9
3	1	0.029	147.139	506.769	92094.01	23	15	9

3	1	0.04	133.862	444.306	80934.42	0	16	9
3	1	0.039	133.883	450.058	81170.33	1	17	9
3	1	0.03	133.899	406.867	81388.34	1	17	9
3	1	0.035	133.917	431.29	81614.53	2	18	9
3	1	0.021	133.928	371.177	81813.21	2	18	9
3	1	0.022	133.94	346.472	81994.91	3	19	9
3	1	0.028	133.954	359.76	82183.39	3	19	9
3	1	0.029	133.97	338.295	82364.47	4	20	9
3	1	0.041	133.991	382.111	82564.87	4	20	9
3	1	0.037	134.011	316.807	82731.02	5	21	9
3	1	0.037	134.03	273.275	82877.22	5	21	9
3	1	0.055	134.059	320.312	83045.2	6	22	9
3	1	0.067	134.095	308.934	83207.22	6	22	9
3	1	0.074	134.133	271.504	83349.61	7	23	9
3	1	0.105	134.189	307.556	83510.91	7	23	9
3	1	0.079	147.314	267.716	95497.77	8	0	10
3	1	0.671	147.68	471.106	95755.05	8	0	10
3	1	8.121	152.119	571.004	96067.2	9	1	10
3	1	24.487	165.22	720.835	96452.84	9	1	10
3	1	39.403	186.322	857.766	96912.23	10	2	10
3	1	36.519	205.89	958.184	97425.66	10	2	10
3	1	27.244	220.473	950.414	97934.39	11	3	10
3	1	19.006	230.657	953.369	98445.23	11	3	10
3	1	14.382	238.372	1013.014	98988.6	12	4	10
3	1	10.625	244.062	997.736	99522.95	13	5	10
3	1	7.958	248.32	980.684	100047.6	13	5	10
3	1	6.23	251.656	991.527	100578.6	14	6	10
3	1	4.137	253.924	844.529	101041.5	14	6	10
3	1	2.727	255.413	767.887	101460.8	15	7	10
3	1	2.343	256.692	844.278	101921.9	15	7	10
3	1	1.697	257.619	780.172	102348	16	8	10
3	1	1.318	258.34	749.628	102757.8	16	8	10
3	1	0.963	258.866	695.184	103137.6	17	9	10
3	1	0.798	259.303	694.414	103517.8	17	9	10
3	1	0.659	259.663	677.391	103887.7	18	10	10
3	1	0.526	259.95	638.844	104237	19	11	10
3	1	0.443	260.193	643.733	104589	19	11	10
3	1	0.184	260.293	435.965	104827.4	20	12	10
3	1	0.154	260.378	461.819	105080.4	20	12	10
3	1	0.186	260.479	535.556	105373.1	21	13	10
3	1	0.195	260.586	591.335	105696.6	21	13	10
3	1	0.137	260.661	552.839	105998.8	22	14	10
3	1	0.103	260.717	540.168	106294.1	22	14	10

3	1	0.073	260.756	506.802	106570.8	23	15	10
3	1	0.061	260.79	491.178	106839.3	23	15	10
3	1	0.027	147.154	503.927	92364.17	0	16	10
3	1	0.014	147.162	493.029	92628.22	0	16	10
3	1	0.009	147.166	484.498	92887.42	1	17	10
3	1	0.004	147.168	466.282	93137.14	1	17	10
3	1	0.003	147.17	458.086	93382.47	2	18	10
3	1	0.001	147.171	423.121	93609.43	2	18	10
3	1	0.004	147.173	415.98	93831.98	3	19	10
3	1	0.007	147.176	415.901	94054.71	3	19	10
3	1	0.012	147.183	398.207	94268.2	4	20	10
3	1	0.009	147.187	378.934	94471.13	5	21	10
3	1	0.016	147.196	363.706	94666.02	5	21	10
3	1	0.023	147.208	369.421	94863.86	6	22	10
3	1	0.029	147.224	339.059	95045.45	6	22	10
3	1	0.026	147.237	285.774	95195.32	7	23	10
3	1	0.063	147.271	296.599	95354.25	7	23	10
3	1	0.338	262.108	286.183	110147.9	8	0	11
3	1	0.35	262.299	87.941	110196	8	0	11
3	1	8.438	266.823	182.097	110293.6	9	1	11
3	1	26.749	281.148	394.384	110504.8	9	1	11
3	1	41.971	303.603	549.187	110798.6	10	2	11
3	1	33.225	321.396	477.616	111054.4	10	2	11
3	1	29.77	337.654	630.529	111398.7	11	3	11
3	1	24.562	351.088	761.02	111815	11	3	11
3	1	16.42	360.078	709.942	112203.7	12	4	11
3	1	11.165	366.185	649.732	112559	13	5	11
3	1	9.7	371.485	784.602	112987.7	13	5	11
3	1	8.068	375.897	859.219	113457.7	14	6	11
3	1	5.91	379.13	789.508	113889.5	14	6	11
3	1	4.592	381.642	805.935	114330.3	15	7	11
3	1	3.471	383.538	806.587	114771	15	7	11
3	1	2.308	384.8	731.899	115171.3	16	8	11
3	1	1.677	385.718	689.913	115548.7	16	8	11
3	1	1.233	386.391	654.436	115906.1	17	9	11
3	1	0.931	386.9	661.167	116267.5	17	9	11
3	1	0.767	387.319	677.757	116637.6	18	10	11
3	1	0.529	387.608	621.604	116977.1	19	11	11
3	1	0.383	387.817	583.065	117296	19	11	11
3	1	0.246	387.952	515.564	117577.6	20	12	11
3	1	0.161	388.04	471.377	117835.5	20	12	11
3	1	0.085	388.086	409.931	118059.7	21	13	11
3	1	0.108	388.145	491.626	118328.6	21	13	11

3	1	0.093	388.196	511.837	118608.7	22	14	11
3	1	0.069	388.234	501.734	118882.7	22	14	11
3	1	0.057	388.265	503.965	119158.3	23	15	11
3	1	0.008	388.269	419.101	119387.6	23	15	11
3	1	0.05	260.817	477.558	107100.3	0	16	11
3	1	0.03	260.834	442.466	107342.2	1	17	11
3	1	0.039	260.855	467.314	107597.6	1	17	11
3	1	0.041	260.877	455.388	107846.7	2	18	11
3	1	0.039	260.899	429.161	108081.4	2	18	11
3	1	0.053	260.928	423.386	108312.9	3	19	11
3	1	0.066	260.964	390.062	108526.2	3	19	11
3	1	0.1	261.019	399.169	108744.5	4	20	11
3	1	0.141	261.096	392.932	108959.6	4	20	11
3	1	0.196	261.203	393.783	109174.6	5	21	11
3	1	0.248	261.339	380.395	109382.3	5	21	11
3	1	0.3	261.502	372.928	109586	6	22	11
3	1	0.358	261.698	375.221	109791.1	7	23	11
3	1	0.411	261.923	366.113	109991.7	7	23	11
3	1	0.201	388.771	269.877	122273.8	8	0	12
3	1	0.196	388.876	227.31	122395.5	8	0	12
3	1	0	388.27	398.131	119605	0	16	12
3	1	0.006	388.273	436.44	119843.6	1	17	12
3	1	0.009	388.278	441.761	120085.1	1	17	12
3	1	0.005	388.281	421.24	120315.5	2	18	12
3	1	0.005	388.284	393.935	120531.5	2	18	12
3	1	0.011	388.29	402.05	120746.8	3	19	12
3	1	0.017	388.299	357.254	120941.9	3	19	12
3	1	0.037	388.319	369.948	121140.3	4	20	12
3	1	0.042	388.342	315.056	121312.6	4	20	12
3	1	0.082	388.386	345.339	121501.2	5	21	12
3	1	0.069	388.423	286.644	121654.9	5	21	12
3	1	0.083	388.467	250.094	121788.9	6	22	12
3	1	0.161	388.554	323.144	121961.9	7	23	12
3	1	0.201	388.661	307.083	122126.2	7	23	12
3	2	0.525	472.803	167.084	54811.82	8	0	8
3	2	0.89	473.289	286.324	54968.27	8	0	8
3	2	0.165	533.419	187.092	61301.91	8	0	8
3	2	0.933	533.93	331.758	61483.54	8	0	8
3	2	1.126	473.904	308.756	55136.88	9	1	8
3	2	5.19	536.767	403.657	61704.21	9	1	8
3	2	13.61	544.199	377.34	61910.28	9	1	8
3	2	2.987	475.504	207.12	55247.81	10	2	8
3	2	10.415	481.197	442.356	55489.63	10	2	8

3	2	27.018	558.961	456.786	62159.86	10	2	8
3	2	15.848	489.852	588.142	55810.82	11	3	8
3	2	18.509	499.97	684.968	56185.27	11	3	8
3	2	40.028	580.866	570.884	62472.26	11	3	8
3	2	47.999	607.092	698.343	62853.82	11	3	8
3	2	16.974	509.24	696.179	56565.46	12	4	8
3	2	12.6	516.128	597.04	56891.84	12	4	8
3	2	39.887	628.919	695.43	63234.38	12	4	8
3	2	27.675	644.04	670.318	63600.63	12	4	8
3	2	8.306	520.666	505.865	57168.24	13	5	8
3	2	7.068	524.53	532.303	57459.23	13	5	8
3	2	19.724	654.823	709.187	63988.32	13	5	8
3	2	12.243	661.519	596.819	64314.75	13	5	8
3	2	4.535	527.007	407.851	57681.96	14	6	8
3	2	1.326	527.731	140.556	57758.72	14	6	8
3	2	7.762	665.762	513.527	64595.47	14	6	8
3	2	6.527	669.336	612.317	64930.71	14	6	8
3	2	0.472	527.984	45.715	57783.23	15	7	8
3	2	4.795	671.957	615.864	65267.39	15	7	8
3	2	0.597	528.304	56.672	57813.58	16	8	8
3	2	0.972	528.835	120.91	57879.71	16	8	8
3	2	3.794	674.032	664.655	65630.91	16	8	8
3	2	2.724	675.52	609.88	65963.98	16	8	8
3	2	0.963	529.351	145.756	57957.77	17	9	8
3	2	1.252	530.02	290.652	58113.27	17	9	8
3	2	1.925	676.573	538.531	66258.38	17	9	8
3	2	1.479	677.382	492.332	66527.66	17	9	8
3	2	1.138	530.63	381.146	58317.4	18	10	8
3	2	0.923	531.124	393.192	58527.98	18	10	8
3	2	1.502	678.203	589.682	66850.02	18	10	8
3	2	1.073	678.789	494.756	67120.21	18	10	8
3	2	0.808	531.567	429.243	58762.75	19	11	8
3	2	0.637	531.908	390.891	58972.09	19	11	8
3	2	0.957	679.312	483.549	67384.95	19	11	8
3	2	0.586	679.632	371.528	67587.85	19	11	8
3	2	0.263	532.049	205.065	59082.09	20	12	8
3	2	0.331	532.226	270.483	59227.1	20	12	8
3	2	0.361	679.83	295.942	67749.72	20	12	8
3	2	0.25	679.964	281.451	67900.45	20	12	8
3	2	0.309	532.391	297.383	59386.28	21	13	8
3	2	0.243	532.524	295.161	59547.8	21	13	8
3	2	0.048	679.989	169.039	67990.98	21	13	8
3	2	0.064	680.023	201.027	68098.53	21	13	8

3	2	0.162	532.613	267.799	59694.13	22	14	8
3	2	0.011	680.029	172.615	68191.07	22	14	8
3	2	0.107	532.671	217.442	59813.11	23	15	8
3	2	0.111	532.732	243.998	59946.36	23	15	8
3	2	0.001	680.03	164.327	68278.98	23	15	8
3	2	-0.001	680.029	142.693	68355.33	23	15	8
3	2	0.072	532.771	194.929	60052.82	0	16	8
3	2	0.029	532.787	125.26	60121.22	0	16	8
3	2	0.027	532.801	51.45	60148.78	1	17	8
3	2	0.059	532.832	95.119	60199.72	1	17	8
3	2	0.11	532.892	257.373	60340.34	2	18	8
3	2	0.09	532.942	204.917	60452.31	2	18	8
3	2	0.069	532.979	182.247	60551.84	3	19	8
3	2	0.068	533.017	176.677	60648.37	3	19	8
3	2	0.063	533.051	162.795	60737.59	4	20	8
3	2	0.069	533.089	151.881	60820.66	5	21	8
3	2	0.074	533.13	156.337	60906.17	5	21	8
3	2	0.072	533.169	127.35	60975.89	6	22	8
3	2	0.043	533.192	75.03	61016.04	6	22	8
3	2	0.118	533.257	182.46	61115.78	7	23	8
3	2	0.131	533.329	153.303	61199.63	7	23	8
3	2	0.027	680.153	41.898	69030.13	8	0	9
3	2	0.244	680.278	234.492	69150.51	8	0	9
3	2	4.401	682.635	501.534	69419.11	9	1	9
3	2	14.453	690.367	428.925	69648.59	9	1	9
3	2	20.083	701.351	370.198	69851.06	10	2	9
3	2	34.118	720.002	510.73	70130.26	10	2	9
3	2	42.369	743.14	623.761	70470.9	11	3	9
3	2	37.241	763.478	634.539	70817.43	11	3	9
3	2	30.688	780.271	620.595	71157.03	12	4	9
3	2	24.526	793.664	672.881	71524.5	12	4	9
3	2	20.527	804.88	725.119	71920.7	13	5	9
3	2	15.524	813.375	716.17	72312.6	13	5	9
3	2	10.021	818.848	609.312	72645.35	14	6	9
3	2	7.206	822.785	617.064	72982.51	15	7	9
3	2	5.156	825.602	627.658	73325.45	15	7	9
3	2	3.324	827.42	577.593	73641.37	16	8	9
3	2	2.16	828.601	524.741	73928.38	16	8	9
3	2	1.569	829.459	513.837	74209.27	17	9	9
3	2	1.169	830.098	498.381	74481.59	17	9	9
3	2	0.929	830.605	489.405	74749.13	18	10	9
3	2	0.641	830.956	421.926	74979.9	18	10	9
3	2	0.503	831.231	400.386	75198.66	19	11	9

3	2	0.449	831.476	395.463	75414.96	19	11	9
3	2	0.308	831.641	348.161	75601.23	20	12	9
3	2	0.177	831.738	298.714	75764.52	21	13	9
3	2	0.111	831.797	292.44	75920.98	21	13	9
3	2	0.012	831.804	216.308	76039.23	22	14	9
3	2	-0.001	831.803	139.269	76113.81	22	14	9
3	2	0	831.803	77.858	76154.6	23	15	9
3	2	0.002	831.804	220.504	76272.81	23	15	9
3	2	0.01	680.034	93.027	68404.12	0	16	9
3	2	-0.001	680.033	139.415	68478.74	0	16	9
3	2	0.008	680.038	74.953	68518.05	1	17	9
3	2	0	680.038	130.31	68587.84	1	17	9
3	2	0.012	680.044	61.868	68620.29	2	18	9
3	2	0.005	680.047	134.398	68692.2	2	18	9
3	2	0.007	680.05	39.363	68712.84	3	19	9
3	2	0.022	680.062	73.06	68751.13	3	19	9
3	2	0.006	680.065	105.314	68807.48	4	20	9
3	2	0.018	680.074	61.084	68839.52	4	20	9
3	2	0.032	680.091	80.116	68881.53	5	21	9
3	2	0.018	680.101	97.573	68933.73	5	21	9
3	2	0.025	680.114	44.612	68957.13	6	22	9
3	2	0.018	680.123	45.522	68981.01	6	22	9
3	2	0.03	680.139	51.735	69008.16	7	23	9
3	2	0.293	832.399	273.922	78221.67	8	0	10
3	2	2.124	833.56	530.075	78511.45	8	0	10
3	2	11.263	839.711	584.232	78830.5	9	1	10
3	2	19.62	850.218	689.679	79199.86	9	1	10
3	2	24.171	863.163	867.182	79664.28	10	2	10
3	2	23.58	875.785	1048.901	80225.73	11	3	10
3	2	17.631	885.227	1032.376	80778.63	11	3	10
3	2	13.077	892.238	1139.889	81389.74	12	4	10
3	2	8.472	896.778	996.523	81923.71	12	4	10
3	2	6.028	900.007	865.056	82387	13	5	10
3	2	5.936	903.184	954.032	82897.67	13	5	10
3	2	4.511	905.601	863.479	83360.35	14	6	10
3	2	3.602	907.573	868.906	83836.08	14	6	10
3	2	3.074	909.252	909.442	84332.73	15	7	10
3	2	2.822	910.793	982.946	84869.53	15	7	10
3	2	1.743	911.746	791.574	85302.26	16	8	10
3	2	1.562	912.599	843.26	85762.77	16	8	10
3	2	1.452	913.392	893.12	86251.02	17	9	10
3	2	1.089	913.988	732.996	86652.13	18	10	10
3	2	1.143	914.613	929.466	87159.98	18	10	10

3	2	0.811	915.056	673.056	87528.1	19	11	10
3	2	0.441	915.297	503.997	87803.48	19	11	10
3	2	0.334	915.48	468.684	88059.95	20	12	10
3	2	0.406	915.702	643.912	88412.49	20	12	10
3	2	0.248	915.837	585.411	88732.68	21	13	10
3	2	0.08	915.881	391.458	88946.68	21	13	10
3	2	0.076	915.923	441.931	89188.15	22	14	10
3	2	0.038	915.944	410.91	89412.78	22	14	10
3	2	0.034	915.962	408.552	89636.13	23	15	10
3	2	-0.002	831.803	202.285	76381.2	0	16	10
3	2	0.004	831.806	276.322	76529.19	0	16	10
3	2	0.001	831.806	270.47	76673.89	1	17	10
3	2	0.006	831.81	273.386	76820.3	1	17	10
3	2	0.003	831.811	267.761	76963.85	2	18	10
3	2	0.009	831.816	251.405	77098.56	2	18	10
3	2	0.016	831.825	232.605	77223.01	3	19	10
3	2	0.047	831.85	247.568	77355.73	4	20	10
3	2	0.073	831.889	246.565	77487.85	4	20	10
3	2	0.086	831.935	222.005	77606.75	5	21	10
3	2	0.119	831.999	234.499	77732.34	5	21	10
3	2	0.114	832.06	197.702	77838.33	6	22	10
3	2	0.055	832.089	129.033	77907.36	6	22	10
3	2	0.093	832.138	97.432	77958.51	7	23	10
3	2	0.195	832.243	217.327	78074.9	7	23	10
3	2	0.079	916.386	167.415	92264.48	8	0	11
3	2	1.563	917.24	70.339	92302.91	8	0	11
3	2	4.24	919.512	61.491	92335.85	9	1	11
3	2	6.3	922.886	62.872	92369.52	9	1	11
3	2	-0.406	922.668	-105.004	92313.29	10	2	11
3	2	3.613	924.601	167.647	92402.98	10	2	11
3	2	27.65	939.701	691.476	92780.6	11	3	11
3	2	20.997	951.197	689.198	93157.94	12	4	11
3	2	13.995	958.859	588.36	93480.06	12	4	11
3	2	9.845	964.241	561.522	93787.03	13	5	11
3	2	8.641	968.963	665.196	94150.48	13	5	11
3	2	7.783	973.217	758.538	94565.15	14	6	11
3	2	6.785	976.932	828.528	95018.77	14	6	11
3	2	5.653	980.022	887.241	95503.79	15	7	11
3	2	4.835	982.663	941.535	96017.97	15	7	11
3	2	3.556	984.608	843.018	96479.05	16	8	11
3	2	2.78	986.129	810.549	96922.38	16	8	11
3	2	2.455	987.471	846.39	97385.08	17	9	11
3	2	1.997	988.561	799.294	97821.58	18	10	11

3	2	1.645	989.46	785.416	98250.5	18	10	11
3	2	1.188	990.109	699.703	98633.01	19	11	11
3	2	1.005	990.658	696.328	99013.66	19	11	11
3	2	0.751	991.069	590.076	99335.91	20	12	11
3	2	0.342	991.256	374.57	99540.89	20	12	11
3	2	0.448	991.501	494.48	99811.48	21	13	11
3	2	0.391	991.715	524.608	100098.4	21	13	11
3	2	0.28	991.868	493.559	100368.2	22	14	11
3	2	0.166	991.958	466.463	100623	22	14	11
3	2	0.098	992.012	434.552	100860.6	23	15	11
3	2	0.042	915.985	438.698	89875.83	0	16	11
3	2	0.017	915.994	397.879	90093.12	0	16	11
3	2	-0.001	915.994	293.838	90253.75	1	17	11
3	2	0.005	915.997	365.51	90453.56	1	17	11
3	2	0.007	916	364.4	90652.87	2	18	11
3	2	0.004	916.003	309.628	90822.22	2	18	11
3	2	0.003	916.004	267.087	90968.23	3	19	11
3	2	0.022	916.016	308.037	91136.88	3	19	11
3	2	0.038	916.037	281.959	91291.02	4	20	11
3	2	0.072	916.076	317.683	91464.77	4	20	11
3	2	0.068	916.114	255.025	91604.05	5	21	11
3	2	0.079	916.157	252.025	91741.68	6	22	11
3	2	0.096	916.209	252.043	91879.46	6	22	11
3	2	0.115	916.272	265.012	92024.41	7	23	11
3	2	0.131	916.343	271.771	92173.05	7	23	11
3	2	0.038	992.162	151.316	103394	8	0	12
3	2	0.193	992.265	372.037	103593.5	8	0	12
3	2	0.039	992.033	377.615	101067.2	0	16	12
3	2	0.011	992.039	345.563	101256.1	0	16	12
3	2	0.005	992.041	345.009	101444.5	1	17	12
3	2	0.005	992.044	366.814	101645.1	1	17	12
3	2	0.001	992.045	371.901	101848.6	2	18	12
3	2	-0.002	992.043	335.657	102032.5	2	18	12
3	2	0.002	992.044	377.347	102234.6	3	19	12
3	2	0.007	992.048	339.094	102420	3	19	12
3	2	0.001	992.048	259.175	102559	4	20	12
3	2	0.008	992.052	275.055	102709.4	4	20	12
3	2	0.034	992.071	297.544	102871.8	5	21	12
3	2	0.009	992.076	206.047	102982.4	6	22	12
3	2	0.006	992.079	162.262	103069.2	6	22	12
3	2	0.051	992.106	227.436	103191	7	23	12
3	2	0.065	992.141	224.81	103311.3	7	23	12
3	3	0.424	309.204	268.951	55296.19	8	0	8

3	3	0.389	309.416	266.046	55441.48	8	0	8
3	3	0.401	334.543	460.489	64016.21	8	0	8
3	3	0.714	309.806	489.593	55709.13	9	1	8
3	3	1.321	310.527	488.785	55976.05	9	1	8
3	3	2.884	336.122	603.108	64346.41	9	1	8
3	3	8.329	340.675	468.754	64602.67	9	1	8
3	3	3.173	312.227	353.705	56165.48	10	2	8
3	3	5.638	315.307	423.442	56396.85	10	2	8
3	3	11.548	346.982	381.119	64810.8	10	2	8
3	3	16.875	356.207	452.697	65058.27	10	2	8
3	3	6.089	318.633	508.333	56674.45	11	3	8
3	3	4.407	321.041	455.725	56923.58	11	3	8
3	3	17.719	365.908	499.974	65332.01	11	3	8
3	3	16.718	375.038	572.092	65644.43	11	3	8
3	3	3.869	323.155	522.689	57209.03	12	4	8
3	3	3.081	324.84	530.034	57498.93	12	4	8
3	3	12.945	382.126	607.174	65976.86	12	4	8
3	3	9.794	387.475	660.83	66337.75	12	4	8
3	3	2.628	326.275	549.161	57798.83	13	5	8
3	3	7.113	391.366	670.656	66704.56	13	5	8
3	3	4.938	394.065	609.753	67037.89	13	5	8
3	3	2.945	327.885	640.041	58148.72	14	6	8
3	3	2.828	329.43	652.91	58505.28	14	6	8
3	3	3.301	395.869	563.274	67345.66	14	6	8
3	3	2.774	330.915	803.334	58935.29	15	7	8
3	3	2.079	332.029	786.719	59356.84	15	7	8
3	3	2.356	397.159	593.186	67670.42	15	7	8
3	3	1.601	398.035	587.974	67992.01	15	7	8
3	3	1.526	332.863	741.95	59762.64	16	8	8
3	3	0.746	333.263	476.038	60017.59	16	8	8
3	3	1.101	398.636	575.323	68306.52	16	8	8
3	3	0.709	399.023	509.564	68584.8	16	8	8
3	3	0.048	333.289	111.459	60078.52	17	9	8
3	3	0.071	333.327	50.768	60105.68	17	9	8
3	3	0.308	399.192	305.857	68752.09	17	9	8
3	3	0.35	399.383	359.864	68949.01	17	9	8
3	3	0.076	333.368	47.584	60131.16	18	10	8
3	3	0.083	333.412	54.016	60160.12	18	10	8
3	3	0.28	399.536	303.453	69114.73	18	10	8
3	3	0.189	399.639	257.384	69255.43	18	10	8
3	3	0.002	333.414	76.136	60201.72	19	11	8
3	3	0.031	333.43	45.413	60226.07	19	11	8
3	3	0.134	399.712	224.622	69378.29	19	11	8

3	3	0.028	399.728	168.867	69470.51	19	11	8
3	3	0.042	333.453	50.682	60253.23	20	12	8
3	3	-0.001	399.727	103.493	69527.11	20	12	8
3	3	0.046	333.477	47.708	60278.8	21	13	8
3	3	0.048	333.503	56.755	60309.2	21	13	8
3	3	0.003	399.729	53.069	69555.53	21	13	8
3	3	0.026	399.743	49.127	69581.84	21	13	8
3	3	0.122	333.57	337.609	60493.95	22	14	8
3	3	0.1	333.625	401.476	60713.31	22	14	8
3	3	0.016	399.751	38.504	69602.45	22	14	8
3	3	0.015	399.759	34.404	69620.89	22	14	8
3	3	0.071	333.663	386.356	60924.63	23	15	8
3	3	0.069	333.701	399.6	61142.85	23	15	8
3	3	0.015	399.768	31.144	69637.55	23	15	8
3	3	0.031	399.784	37.616	69657.27	23	15	8
3	3	0.035	333.72	306.077	61310	0	16	8
3	3	0.027	333.734	268.096	61453.43	0	16	8
3	3	0.046	333.759	343.081	61637.17	1	17	8
3	3	0.059	333.791	374.346	61841.81	1	17	8
3	3	0.059	333.823	366.504	62042.17	2	18	8
3	3	0.06	333.856	364.217	62241.07	2	18	8
3	3	0.065	333.892	365.082	62440.55	3	19	8
3	3	0.06	333.925	345.774	62629.57	4	20	8
3	3	0.059	333.957	336.757	62814.13	4	20	8
3	3	0.073	333.997	336.831	62998.36	5	21	8
3	3	0.079	334.04	322.628	63174.82	5	21	8
3	3	0.066	334.076	250.441	63309.09	6	22	8
3	3	0.1	334.13	246.274	63443.58	6	22	8
3	3	0.156	334.216	289.763	63602.06	7	23	8
3	3	0.198	334.324	297.336	63764.61	7	23	8
3	3	0.027	399.993	13.24	69839.51	8	0	9
3	3	0.051	400.02	18.831	69849.38	8	0	9
3	3	0.156	400.103	19.388	69859.77	9	1	9
3	3	0.615	400.433	191.412	69962.28	9	1	9
3	3	3.201	402.182	288.282	70119.88	10	2	9
3	3	6.392	405.675	202.303	70230.41	10	2	9
3	3	8.751	410.454	208.33	70344.19	11	3	9
3	3	9.038	415.39	231.937	70470.85	11	3	9
3	3	7.959	419.745	238.883	70601.57	12	4	9
3	3	6.771	423.444	294.215	70762.33	13	5	9
3	3	5.43	426.411	336.711	70946.3	13	5	9
3	3	3.653	428.409	314.36	71118.24	14	6	9
3	3	2.165	429.592	265.526	71263.25	14	6	9

3	3	1.23	430.264	225.275	71386.4	15	7	9
3	3	0.874	430.742	237.712	71516.28	15	7	9
3	3	0.637	431.091	251.094	71653.69	16	8	9
3	3	0.368	431.291	227.109	71777.77	16	8	9
3	3	0.262	431.435	234.156	71905.91	17	9	9
3	3	0.173	431.529	240.721	72037.37	17	9	9
3	3	0.088	431.577	221.694	72158.5	18	10	9
3	3	0.019	431.588	190.138	72262.55	19	11	9
3	3	0.02	431.598	200.148	72371.85	19	11	9
3	3	-0.001	431.598	142.245	72449.65	20	12	9
3	3	0	431.598	58.135	72480.75	20	12	9
3	3	-0.001	431.597	139.234	72556.87	21	13	9
3	3	0	431.597	54.676	72586.12	21	13	9
3	3	-0.001	431.596	114.065	72648.48	22	14	9
3	3	0	431.596	49.439	72674.41	22	14	9
3	3	0	431.596	35.233	72693.26	23	15	9
3	3	0	431.596	33.972	72711.48	23	15	9
3	3	0.019	399.794	31.004	69673.87	0	16	9
3	3	0.018	399.803	23.567	69686.22	0	16	9
3	3	0.019	399.814	28.79	69701.65	1	17	9
3	3	0.021	399.825	21.222	69712.77	1	17	9
3	3	0.023	399.837	26.642	69727.04	2	18	9
3	3	0.021	399.848	18.049	69736.49	2	18	9
3	3	0.037	399.868	31.102	69752.8	3	19	9
3	3	0.026	399.882	24.527	69765.93	3	19	9
3	3	0.023	399.893	19.656	69776.23	4	20	9
3	3	0.027	399.908	17.53	69785.42	4	20	9
3	3	0.028	399.922	22.9	69797.69	5	21	9
3	3	0.026	399.936	17.616	69806.92	6	22	9
3	3	0.026	399.949	15.847	69815.23	6	22	9
3	3	0.028	399.964	18.778	69825.09	7	23	9
3	3	0.028	399.979	14.253	69832.56	7	23	9
3	3	0.128	431.795	159.8	73080.63	8	0	10
3	3	0.702	432.179	293.949	73241.32	8	0	10
3	3	1.54	433.02	126.778	73310.55	9	1	10
3	3	0.314	433.188	33.781	73328.65	10	2	10
3	3	0.014	433.196	52.675	73356.88	10	2	10
3	3	0.1	433.249	33.732	73374.93	11	3	10
3	3	0.739	433.645	33.433	73392.84	11	3	10
3	3	0.353	433.835	32.151	73410.08	12	4	10
3	3	0.441	434.071	42.796	73433	12	4	10
3	3	0.439	434.306	44.083	73456.59	13	5	10
3	3	0.378	434.508	37.201	73476.52	13	5	10

3	3	0.536	434.795	63.709	73510.63	14	6	10
3	3	1.403	435.563	508.881	73789.24	14	6	10
3	3	1.386	436.32	827.017	74240.88	15	7	10
3	3	1.181	436.965	909.735	74737.7	15	7	10
3	3	1.129	437.583	952.958	75258.66	16	8	10
3	3	1.007	438.133	927.156	75764.98	17	9	10
3	3	0.907	438.628	912.914	76264.3	17	9	10
3	3	0.801	439.067	893.18	76753.06	18	10	10
3	3	0.646	439.42	840.989	77212.57	18	10	10
3	3	0.508	439.698	794.693	77647.45	19	11	10
3	3	0.263	439.842	597.482	77973.73	19	11	10
3	3	0.152	439.925	523.751	78260.48	20	12	10
3	3	0.115	439.988	556.19	78564.69	20	12	10
3	3	0.083	440.034	616.667	78902.31	21	13	10
3	3	0.037	440.054	593.198	79226.43	21	13	10
3	3	0.008	440.059	582.362	79544.46	22	14	10
3	3	-0.002	440.058	545.131	79842.47	23	15	10
3	3	-0.003	440.056	513.298	80123.07	23	15	10
3	3	0.001	431.596	34.591	72730	0	16	10
3	3	0	431.596	35.526	72749.02	0	16	10
3	3	0.003	431.598	33.995	72767.21	1	17	10
3	3	0.006	431.601	37.349	72787.21	2	18	10
3	3	0.009	431.606	37.11	72807.12	2	18	10
3	3	0.008	431.61	37.844	72827.38	3	19	10
3	3	0.016	431.619	35.636	72846.46	3	19	10
3	3	0.019	431.629	39.905	72867.84	4	20	10
3	3	0.02	431.64	38.186	72888.31	4	20	10
3	3	0.028	431.655	36.425	72907.81	5	21	10
3	3	0.017	431.664	36.604	72927.41	5	21	10
3	3	0.025	431.677	31.046	72944.05	6	22	10
3	3	0.012	431.683	24.96	72957.13	6	22	10
3	3	0.044	431.707	36.081	72976.48	7	23	10
3	3	0.037	431.727	34.662	72995.05	7	23	10
3	3	0.316	440.594	467.747	83497.69	8	0	11
3	3	2.901	442.146	522.902	83777.44	8	0	11
3	3	11.565	448.343	377.796	83979.88	9	1	11
3	3	12.212	454.883	370.867	84178.49	9	1	11
3	3	10.678	460.602	398.177	84391.73	10	2	11
3	3	8.368	465.171	440.155	84632.11	11	3	11
3	3	7.068	469.035	496.159	84903.34	11	3	11
3	3	4.826	471.678	487.452	85170.23	12	4	11
3	3	3.361	473.518	462.278	85423.32	12	4	11
3	3	2.889	475.095	508.274	85700.89	13	5	11

3	3	2.674	476.557	622.57	86041.23	13	5	11
3	3	1.868	477.579	596.862	86367.68	14	6	11
3	3	1.275	478.277	559.904	86674.23	14	6	11
3	3	0.911	478.775	521.482	86959.02	15	7	11
3	3	0.679	479.145	527.353	87247.01	15	7	11
3	3	0.469	479.402	469.425	87503.76	16	8	11
3	3	0.336	479.586	462.247	87756.58	17	9	11
3	3	0.25	479.722	424.085	87988.41	17	9	11
3	3	0.189	479.825	420.189	88217.88	18	10	11
3	3	0.122	479.892	400.725	88436.73	18	10	11
3	3	0.074	479.932	378.842	88644.04	19	11	11
3	3	0.032	479.95	336.919	88828.03	19	11	11
3	3	-0.002	479.949	179.186	88925.94	20	12	11
3	3	-0.002	479.948	182.81	89025.98	20	12	11
3	3	-0.003	479.946	271.698	89174.73	21	13	11
3	3	-0.002	479.945	243.256	89307.78	22	14	11
3	3	-0.003	479.944	258.436	89448.91	22	14	11
3	3	-0.002	479.942	225.103	89571.84	23	15	11
3	3	-0.002	479.941	239.302	89702.73	23	15	11
3	3	-0.004	440.054	472.452	80381.34	0	16	11
3	3	-0.004	440.052	467.166	80636.47	0	16	11
3	3	-0.003	440.05	422.588	80867.37	1	17	11
3	3	-0.004	440.048	375.673	81072.95	1	17	11
3	3	-0.003	440.047	411.094	81297.79	2	18	11
3	3	-0.003	440.045	397.552	81515.12	2	18	11
3	3	0	440.045	381.026	81723.3	3	19	11
3	3	0.003	440.047	368.924	81925.29	4	20	11
3	3	0.012	440.053	353.245	82118.49	4	20	11
3	3	0.029	440.069	361.477	82316.1	5	21	11
3	3	0.063	440.103	357.449	82511.31	5	21	11
3	3	0.093	440.154	326.66	82689.7	6	22	11
3	3	0.129	440.225	334.197	82872.4	6	22	11
3	3	0.169	440.317	356.484	83067.57	7	23	11
3	3	0.19	440.421	319.517	83242.24	7	23	11
3	3	0	479.936	84.263	90720.23	8	0	12
3	3	0.209	480.048	319.965	90891.68	8	0	12
3	3	-0.002	479.94	186.324	89804.69	0	16	12
3	3	-0.002	479.939	175.363	89900.5	0	16	12
3	3	-0.002	479.938	162.45	89989.22	1	17	12
3	3	-0.002	479.937	163.741	90078.82	1	17	12
3	3	-0.002	479.936	161.535	90167.3	2	18	12
3	3	-0.001	479.936	148.554	90248.55	2	18	12
3	3	-0.001	479.935	86.509	90294.88	3	19	12

3	3	-0.001	479.934	147.981	90375.86	4	20	12
3	3	-0.001	479.934	77.464	90417.41	4	20	12
3	3	-0.002	479.933	170.048	90510.27	5	21	12
3	3	-0.001	479.932	107.428	90568.97	5	21	12
3	3	0	479.932	34.17	90587.29	6	22	12
3	3	-0.001	479.932	58.931	90618.82	6	22	12
3	3	0.001	479.933	44.869	90642.85	7	23	12
3	3	0.006	479.936	58.485	90674.17	7	23	12
3	4	0.32	399.364	329.996	42692.07	8	0	8
3	4	0.258	399.505	302.149	42857.07	8	0	8
3	4	0.216	425.344	327.321	53383.11	8	0	8
3	4	0.347	425.534	303.032	53548.76	8	0	8
3	4	0.654	399.862	599.76	43184.94	9	1	8
3	4	0.869	400.337	218.315	43304.23	9	1	8
3	4	3.121	427.241	205.56	53661.19	9	1	8
3	4	6.717	430.913	275.263	53811.67	9	1	8
3	4	3.221	402.062	190.372	43406.18	10	2	8
3	4	4.225	404.369	196.237	43513.35	10	2	8
3	4	9.793	436.261	348.667	54002.08	10	2	8
3	4	7.457	440.339	230.952	54128.39	10	2	8
3	4	6.772	408.068	550.065	43813.75	11	3	8
3	4	6.412	411.573	735.747	44215.95	11	3	8
3	4	6.436	443.862	336.84	54312.72	11	3	8
3	4	4.823	446.497	423.261	54543.99	11	3	8
3	4	5	414.304	719.178	44608.7	12	4	8
3	4	2.772	448.014	339.316	54729.67	12	4	8
3	4	2.534	449.399	499.031	55002.33	12	4	8
3	4	3.62	416.284	672.89	44976.92	13	5	8
3	4	3.729	418.321	852.143	45442.29	13	5	8
3	4	2.1	450.548	521.333	55287.62	13	5	8
3	4	2.887	419.898	785.589	45871.52	14	6	8
3	4	2.177	421.087	757.071	46284.97	14	6	8
3	4	1.242	451.226	422.124	55518.26	14	6	8
3	4	0.947	451.744	515.404	55800.02	14	6	8
3	4	1.681	421.987	801.315	46714.12	15	7	8
3	4	1.249	422.657	824.682	47155.78	15	7	8
3	4	0.534	452.036	453.325	56047.96	15	7	8
3	4	0.257	452.177	358.059	56243.9	15	7	8
3	4	0.795	423.091	704.911	47541.33	16	8	8
3	4	0.607	423.417	688.847	47910.63	16	8	8
3	4	0.253	452.315	559.165	56549.42	16	8	8
3	4	0.178	452.411	530.101	56838.91	16	8	8
3	4	0.495	423.681	675.137	48271.82	17	9	8

3	4	0.484	423.941	705.387	48649.4	17	9	8
3	4	0.122	452.478	467.106	57094.52	17	9	8
3	4	0.095	452.53	473.955	57353.75	17	9	8
3	4	0.373	424.14	662.614	49004.09	18	10	8
3	4	0.287	424.297	632.789	49350.36	18	10	8
3	4	0.043	452.553	438.113	57593.01	18	10	8
3	4	0.018	452.563	460.277	57844.75	18	10	8
3	4	0.216	424.413	584.102	49663.18	19	11	8
3	4	-0.003	452.562	399.795	58063.31	19	11	8
3	4	0.029	424.429	280.356	49813.41	20	12	8
3	4	0.048	424.455	393.553	50024.39	20	12	8
3	4	-0.002	452.561	237.672	58193.11	20	12	8
3	4	-0.003	452.559	297.734	58352.72	20	12	8
3	4	0.032	424.472	414.825	50246.67	21	13	8
3	4	0.025	424.485	385.544	50457.54	21	13	8
3	4	-0.003	452.557	321.065	58524.58	21	13	8
3	4	-0.004	452.555	273.333	58670.97	21	13	8
3	4	0.023	424.498	392.735	50672.46	22	14	8
3	4	0.014	424.505	342.652	50859.68	22	14	8
3	4	-0.004	452.553	286.8	58824.48	22	14	8
3	4	-0.005	452.55	306.987	58988.98	22	14	8
3	4	0.014	424.513	342.679	51047.01	23	15	8
3	4	0.008	424.517	338.868	51232.07	23	15	8
3	4	-0.005	452.548	315.381	59157.71	23	15	8
3	4	-0.005	452.545	284.839	59307.01	23	15	8
3	4	-0.001	424.517	160.707	51319.84	0	16	8
3	4	0.011	424.523	315.529	51488.82	0	16	8
3	4	0.019	424.533	312.444	51655.98	1	17	8
3	4	0.026	424.547	293.703	51816.54	2	18	8
3	4	0.034	424.565	293.061	51976.74	2	18	8
3	4	0.046	424.591	298.681	52139.86	3	19	8
3	4	0.071	424.63	249.853	52276.44	3	19	8
3	4	0.097	424.683	210.392	52391.52	4	20	8
3	4	0.123	424.75	219.587	52511.74	4	20	8
3	4	0.154	424.834	258.138	52652.93	5	21	8
3	4	0.16	424.922	216.71	52771.57	5	21	8
3	4	0.129	424.991	183.993	52870.11	6	22	8
3	4	0.208	425.105	293.267	53030.35	6	22	8
3	4	0.221	425.226	317.805	53204.26	7	23	8
3	4	0.066	452.897	109.119	61197.39	8	0	9
3	4	2.063	453.98	551.462	61486.91	8	0	9
3	4	9.444	459.033	412.689	61707.7	9	1	9
3	4	12.449	465.845	245.562	61842.07	9	1	9

3	4	19.855	476.694	277.317	61993.6	10	2	9
3	4	24.849	490.264	385.149	62203.93	10	2	9
3	4	27.276	505.167	546.375	62502.46	11	3	9
3	4	23.986	518.266	652.797	62858.96	12	4	9
3	4	17.769	527.985	697	63240.18	12	4	9
3	4	13.597	535.418	778.714	63665.88	13	5	9
3	4	8.589	540.111	787.697	64096.27	13	5	9
3	4	4.872	542.774	760.737	64512.14	14	6	9
3	4	2.292	544.026	570.396	64823.64	14	6	9
3	4	1.629	544.916	657.038	65182.82	15	7	9
3	4	1.199	545.572	694.912	65562.52	15	7	9
3	4	0.799	546.009	660.443	65923.92	16	8	9
3	4	0.647	546.362	670.682	66290.38	16	8	9
3	4	0.516	546.644	656.388	66649.56	17	9	9
3	4	0.406	546.866	665.631	67013.26	18	10	9
3	4	0.261	547.009	625.255	67354.89	18	10	9
3	4	0.112	547.07	536.656	67648.41	19	11	9
3	4	0.036	547.09	507.734	67925.7	19	11	9
3	4	-0.004	547.088	435.58	68159.09	20	12	9
3	4	-0.004	547.085	440.821	68399.83	20	12	9
3	4	-0.004	547.083	449.588	68640.61	21	13	9
3	4	-0.004	547.081	427.809	68874.24	21	13	9
3	4	-0.002	547.08	210.279	68986.86	22	14	9
3	4	-0.003	547.078	319.427	69154.38	22	14	9
3	4	-0.003	547.076	349.457	69341.44	23	15	9
3	4	-0.002	547.075	252.772	69477.02	23	15	9
3	4	-0.005	452.542	286.703	59460.56	0	16	9
3	4	-0.005	452.539	275.289	59604.78	0	16	9
3	4	-0.005	452.537	255.212	59741.6	1	17	9
3	4	-0.005	452.534	253.494	59874.4	1	17	9
3	4	-0.005	452.531	223.98	59994.36	2	18	9
3	4	-0.005	452.529	225.144	60112.37	2	18	9
3	4	-0.002	452.528	219.87	60227.68	3	19	9
3	4	0.015	452.535	226.571	60348.89	4	20	9
3	4	0.04	452.556	219.831	60464.12	4	20	9
3	4	0.065	452.591	225.498	60582.38	5	21	9
3	4	0.08	452.633	215.786	60697.95	5	21	9
3	4	0.099	452.685	216.408	60811.32	6	22	9
3	4	0.108	452.742	213.034	60923.04	6	22	9
3	4	0.116	452.803	211.292	61033.86	7	23	9
3	4	0.115	452.863	202.706	61140.16	7	23	9
3	4	1.189	548.146	597.666	72230.1	8	0	10
3	4	9.895	553.555	582.259	72548.41	9	1	10

3	4	16.963	562.63	594.682	72866.56	9	1	10
3	4	16.339	571.381	653.883	73216.75	10	2	10
3	4	12.124	577.881	735.925	73611.29	10	2	10
3	4	8.884	582.634	792.387	74035.22	11	3	10
3	4	6.644	586.192	802.708	74465.12	11	3	10
3	4	5.32	589.046	848.848	74920.43	12	4	10
3	4	4.398	591.401	892.15	75398.23	12	4	10
3	4	3.284	593.16	902.387	75881.51	13	5	10
3	4	2.225	594.351	872.138	76348.59	13	5	10
3	4	1.126	594.966	669.894	76714.61	14	6	10
3	4	1.009	595.519	838.835	77173.88	15	7	10
3	4	0.715	595.91	811.019	77616.78	15	7	10
3	4	0.525	596.196	835.264	78072.93	16	8	10
3	4	0.414	596.423	767.644	78492.58	16	8	10
3	4	0.3	596.587	741.634	78897.59	17	9	10
3	4	0.21	596.701	705.675	79283.95	17	9	10
3	4	0.111	596.762	673.137	79651.94	18	10	10
3	4	0.055	596.792	636.718	79999.84	18	10	10
3	4	0.01	596.797	559.496	80306.01	19	11	10
3	4	-0.003	596.796	294.252	80466.78	19	11	10
3	4	-0.005	596.793	469.175	80723.66	20	12	10
3	4	-0.005	596.791	489.195	80991.09	21	13	10
3	4	-0.005	596.788	474.219	81250.72	21	13	10
3	4	-0.004	596.786	434.058	81488	22	14	10
3	4	-0.004	596.783	455.986	81737.02	22	14	10
3	4	-0.004	596.781	434.057	81974.18	23	15	10
3	4	-0.004	596.779	418.525	82202.98	23	15	10
3	4	-0.003	547.073	328.9	69653.16	0	16	10
3	4	-0.003	547.071	315.077	69821.91	1	17	10
3	4	-0.004	547.069	337.806	70002.63	1	17	10
3	4	-0.004	547.067	336.506	70182.85	2	18	10
3	4	-0.004	547.065	308.849	70348.52	2	18	10
3	4	-0.004	547.063	312.536	70515.9	3	19	10
3	4	-0.001	547.063	314.131	70683.96	3	19	10
3	4	0.023	547.075	313.404	70852.07	4	20	10
3	4	0.06	547.107	323.91	71025.54	4	20	10
3	4	0.093	547.157	318.695	71196.22	5	21	10
3	4	0.116	547.22	309.596	71362.02	5	21	10
3	4	0.066	547.255	197.665	71467.94	6	22	10
3	4	0.126	547.321	272.793	71610.93	6	22	10
3	4	0.149	547.401	271.528	71756.57	7	23	10
3	4	0.178	547.497	274.445	71903.55	7	23	10
3	4	0.449	597.563	213.306	85103.03	8	0	11

3	4	8.073	601.887	351.795	85291.44	9	1	11
3	4	12.979	608.838	505.292	85562.05	9	1	11
3	4	10.51	614.463	630.541	85899.56	10	2	11
3	4	6.08	617.72	670.741	86258.78	10	2	11
3	4	3.594	619.682	755.077	86671.14	11	3	11
3	4	2.611	621.111	881.277	87153.4	11	3	11
3	4	1.588	621.98	823.887	87604.48	12	4	11
3	4	1.082	622.572	783.572	88033.05	12	4	11
3	4	1.137	623.193	927.716	88539.68	13	5	11
3	4	0.907	623.689	987.153	89079.6	13	5	11
3	4	0.448	623.935	865.336	89553.13	14	6	11
3	4	0.343	624.122	913.807	90052.94	15	7	11
3	4	0.208	624.236	895.936	90542.22	15	7	11
3	4	0.093	624.287	784.11	90970.87	16	8	11
3	4	0.046	624.312	771.586	91392.88	16	8	11
3	4	0.016	624.32	728.625	91791.2	17	9	11
3	4	-0.004	624.318	688.073	92167.15	17	9	11
3	4	-0.006	624.315	631.868	92512.22	18	10	11
3	4	-0.006	624.312	629.14	92855.8	18	10	11
3	4	-0.006	624.308	622.259	93196.31	19	11	11
3	4	-0.004	624.306	452.141	93443.23	19	11	11
3	4	-0.003	624.304	284.374	93598.7	20	12	11
3	4	-0.004	624.302	399.242	93817.17	21	13	11
3	4	-0.003	624.301	308.046	93985.74	21	13	11
3	4	-0.003	624.299	263.894	94130.08	22	14	11
3	4	-0.003	624.298	272.797	94279.05	22	14	11
3	4	-0.003	624.296	264.595	94423.7	23	15	11
3	4	-0.002	624.295	178.16	94521.15	23	15	11
3	4	-0.004	596.777	388.928	82415.59	0	16	11
3	4	-0.004	596.775	368.598	82616.99	0	16	11
3	4	-0.004	596.773	384.396	82827.02	1	17	11
3	4	-0.004	596.771	359.146	83023.55	1	17	11
3	4	-0.003	596.769	357.118	83218.98	2	18	11
3	4	-0.002	596.767	327.627	83397.98	3	19	11
3	4	0.008	596.772	334.38	83580.78	3	19	11
3	4	0.042	596.795	337.551	83765.41	4	20	11
3	4	0.072	596.834	318.377	83939.63	4	20	11
3	4	0.105	596.891	323.089	84116.16	5	21	11
3	4	0.12	596.957	307.671	84284.19	5	21	11
3	4	0.143	597.034	306.197	84451.41	6	22	11
3	4	0.163	597.123	321.163	84626.98	6	22	11
3	4	0.163	597.212	313.14	84798.59	7	23	11
3	4	0.193	597.318	344.156	84986.54	7	23	11

3	4	0.092	624.539	232.925	96381.7	8	0	12
3	4	1.204	625.197	518.515	96665.3	8	0	12
3	4	-0.002	624.294	220.162	94641.5	0	16	12
3	4	-0.002	624.293	210.873	94756.72	0	16	12
3	4	-0.002	624.292	209.698	94871.3	1	17	12
3	4	-0.002	624.291	228.649	94996.42	1	17	12
3	4	-0.002	624.29	204.441	95108.47	2	18	12
3	4	-0.002	624.289	189.243	95209.82	3	19	12
3	4	-0.002	624.288	192.956	95315.25	3	19	12
3	4	0	624.287	186.856	95415.42	4	20	12
3	4	0.007	624.291	182.455	95515.31	4	20	12
3	4	0.037	624.311	209.867	95629.92	5	21	12
3	4	0.045	624.335	186.025	95729.55	5	21	12
3	4	0.017	624.344	163.456	95817.13	6	22	12
3	4	0.068	624.381	250.678	95951.31	6	22	12
3	4	0.088	624.428	272.507	96097.18	7	23	12
3	4	0.114	624.49	292.197	96257.08	7	23	12
4	1	0.037	689.133	279.975	97718.95	8	0	8
4	1	1.455	689.929	589.89	98041.42	8	0	8
4	1	-0.002	782.33	196.851	112722.3	8	0	8
4	1	0.385	782.536	98.415	112775	8	0	8
4	1	8.685	694.671	701.532	98424.54	9	1	8
4	1	15.984	703.401	864.006	98896.38	9	1	8
4	1	1.686	783.44	74.955	112815.2	9	1	8
4	1	3.313	785.212	130.487	112885	9	1	8
4	1	22.316	715.6	1081.291	99487.49	10	2	8
4	1	16.411	794.211	674.97	113255.1	10	2	8
4	1	24.077	728.769	1251.289	100171.9	11	3	8
4	1	20.308	739.859	1251.282	100855.2	11	3	8
4	1	11.55	800.528	557.558	113560.1	11	3	8
4	1	13.545	807.94	899.847	114052.5	11	3	8
4	1	15.579	748.38	1195.616	101509.1	12	4	8
4	1	11.276	754.541	1014.17	102063.3	12	4	8
4	1	11.04	813.969	1088.975	114647.2	12	4	8
4	1	8.263	818.488	1090.383	115243.6	12	4	8
4	1	11.652	760.914	1179.403	102708.4	13	5	8
4	1	11.477	767.191	1261.198	103398.2	13	5	8
4	1	7.346	822.5	1226.251	115913.3	13	5	8
4	1	6.577	826.092	1186.989	116561.5	13	5	8
4	1	9.473	772.372	1215.757	104063.1	14	6	8
4	1	6.757	776.066	1149.039	104691.3	14	6	8
4	1	5.311	828.996	1144.358	117187.4	14	6	8
4	1	3.807	831.076	1111.03	117794.4	14	6	8

4	1	4.838	778.711	1217.026	105356.6	15	7	8
4	1	2.428	832.403	1068.055	118378	15	7	8
4	1	1.388	833.161	960.968	118902.8	15	7	8
4	1	2.864	780.279	1062.361	105938.2	16	8	8
4	1	1.669	781.192	992.478	106481	16	8	8
4	1	0.697	833.541	822.764	119352.1	16	8	8
4	1	1.029	781.755	937.901	106994	17	9	8
4	1	0.595	782.08	860.618	107464.7	17	9	8
4	1	0.463	833.794	836.204	119809.3	17	9	8
4	1	0.278	833.947	807.812	120251.1	17	9	8
4	1	0.386	782.287	832.437	107910.3	18	10	8
4	1	0.159	782.374	657.227	108269.2	18	10	8
4	1	0.127	834.016	739.892	120655.1	18	10	8
4	1	0.024	834.029	711.924	121044.1	18	10	8
4	1	-0.004	782.372	398.88	108483	19	11	8
4	1	-0.006	782.369	538.625	108777.1	19	11	8
4	1	-0.007	834.025	648.269	121398.3	19	11	8
4	1	-0.006	834.022	575.195	121712.8	19	11	8
4	1	-0.005	782.366	540.449	109072.5	20	12	8
4	1	-0.004	782.363	446.102	109316.5	20	12	8
4	1	-0.005	834.019	504.027	121988.4	20	12	8
4	1	-0.004	834.017	397.08	122205.5	20	12	8
4	1	-0.005	782.361	509.928	109595.3	21	13	8
4	1	-0.003	834.015	339.547	122391.2	21	13	8
4	1	-0.004	834.013	394.195	122606.8	21	13	8
4	1	-0.005	782.358	476.921	109856	22	14	8
4	1	-0.004	782.356	435.751	110094	22	14	8
4	1	-0.005	834.01	456.889	122856.6	22	14	8
4	1	-0.004	782.354	387.696	110305.7	23	15	8
4	1	-0.004	782.352	381.091	110514	23	15	8
4	1	-0.004	834.008	431.79	123092.4	23	15	8
4	1	-0.004	834.006	395.536	123308.6	23	15	8
4	1	-0.002	782.351	171.392	110607.8	0	16	8
4	1	-0.002	782.35	202.396	110716.2	0	16	8
4	1	-0.003	782.348	322.502	110892.4	1	17	8
4	1	-0.003	782.346	315.58	111065.3	1	17	8
4	1	-0.003	782.345	320.323	111240.2	2	18	8
4	1	-0.003	782.343	290.218	111398.9	2	18	8
4	1	-0.003	782.341	328.212	111578.1	3	19	8
4	1	-0.003	782.34	284.951	111734	4	20	8
4	1	-0.003	782.338	256.832	111874.2	4	20	8
4	1	-0.003	782.337	263.583	112018.3	5	21	8
4	1	-0.003	782.335	270.188	112166.1	5	21	8

4	1	-0.002	782.334	249.153	112302.5	6	22	8
4	1	-0.002	782.333	211.46	112415.6	6	22	8
4	1	-0.001	782.332	151.86	112498.6	7	23	8
4	1	-0.002	782.331	221.007	112616.9	7	23	8
4	1	-0.002	833.978	179.398	126154.9	8	0	9
4	1	1.154	834.609	635.543	126502.2	8	0	9
4	1	11.91	841.116	913.607	127001.4	9	1	9
4	1	19.746	851.9	996.579	127545.6	10	2	9
4	1	20.136	862.913	1045.817	128117.6	10	2	9
4	1	17.724	872.607	1222.066	128786	11	3	9
4	1	12.823	879.617	1307.218	129500.6	11	3	9
4	1	8.081	884.034	1222.255	130168.8	12	4	9
4	1	5.734	887.167	1195.708	130822.1	12	4	9
4	1	5.69	890.281	1317.639	131543.2	13	5	9
4	1	5.298	893.174	1324.201	132266.3	13	5	9
4	1	3.952	895.335	1171.791	132907.2	14	6	9
4	1	2.644	896.782	1087.589	133502.1	14	6	9
4	1	1.955	897.85	1141.902	134126.3	15	7	9
4	1	1.294	898.557	1073.596	134712.6	16	8	9
4	1	0.905	899.051	1026.587	135273.8	16	8	9
4	1	0.636	899.399	963.147	135800	17	9	9
4	1	0.451	899.645	900.954	136292.1	17	9	9
4	1	0.295	899.806	859.886	136761.9	18	10	9
4	1	0.169	899.899	812.175	137205.7	18	10	9
4	1	0.095	899.951	799.987	137643	19	11	9
4	1	0.007	899.955	734.259	138044.4	19	11	9
4	1	-0.007	899.951	660.76	138405.8	20	12	9
4	1	-0.006	899.948	580.295	138723	20	12	9
4	1	-0.006	899.945	590.455	139045.6	21	13	9
4	1	-0.006	899.941	595.715	139371.3	22	14	9
4	1	-0.005	899.939	471.268	139628.9	22	14	9
4	1	-0.003	899.937	296.893	139791.1	23	15	9
4	1	-0.005	899.935	466.516	140046	23	15	9
4	1	-0.004	834.004	380.1	123516.2	0	16	9
4	1	-0.004	834.002	370.692	123718.8	0	16	9
4	1	-0.003	834	352.661	123911.5	1	17	9
4	1	-0.003	833.998	335.232	124094.8	1	17	9
4	1	-0.003	833.996	342.09	124281.6	2	18	9
4	1	-0.003	833.995	335.549	124464.9	2	18	9
4	1	-0.003	833.993	346.52	124654.3	3	19	9
4	1	-0.003	833.991	335.528	124837.5	3	19	9
4	1	-0.003	833.989	325.889	125015.5	4	20	9
4	1	-0.003	833.987	320.633	125190.9	5	21	9

4	1	-0.003	833.986	310.22	125360.4	5	21	9
4	1	-0.003	833.984	307.685	125528.6	6	22	9
4	1	-0.003	833.982	336.511	125712.7	6	22	9
4	1	-0.003	833.98	327.006	125891.5	7	23	9
4	1	-0.003	833.979	302.395	126056.9	7	23	9
4	1	0.508	900.203	560.113	143533.1	8	0	10
4	1	7.871	904.506	903.525	144027	8	0	10
4	1	18.942	914.861	1162.761	144662.6	9	1	10
4	1	21.38	926.542	1348.038	145399.2	10	2	10
4	1	16.701	935.663	1342.352	146132.3	10	2	10
4	1	12.013	942.23	1377.355	146885.2	11	3	10
4	1	8.553	946.906	1474.308	147691.2	11	3	10
4	1	6.026	950.202	1472.163	148496.3	12	4	10
4	1	5.171	953.026	1498.735	149314.8	12	4	10
4	1	4.765	955.631	1517.638	150144.5	13	5	10
4	1	4.155	957.9	1500.034	150963.6	13	5	10
4	1	3.305	959.705	1451.258	151756.2	14	6	10
4	1	2.324	960.974	1358.514	152498.1	14	6	10
4	1	1.552	961.824	1263.217	153189.7	15	7	10
4	1	1.004	962.372	1162.36	153824.5	16	8	10
4	1	0.614	962.708	1064.409	154406.4	16	8	10
4	1	0.41	962.932	1008.458	154957.9	17	9	10
4	1	0.278	963.084	1028.171	155520	17	9	10
4	1	0.114	963.146	810.901	155963.5	18	10	10
4	1	0.037	963.167	754.451	156376	18	10	10
4	1	-0.006	963.164	573.884	156689.8	19	11	10
4	1	-0.004	963.162	377.677	156896.3	19	11	10
4	1	-0.005	963.159	521.659	157181.6	20	12	10
4	1	-0.006	963.156	571.111	157493.8	20	12	10
4	1	-0.005	963.153	522.253	157779.2	21	13	10
4	1	-0.005	963.15	483.307	158043.8	22	14	10
4	1	-0.005	963.148	478.393	158305	22	14	10
4	1	-0.004	963.145	427.325	158538.6	23	15	10
4	1	-0.004	963.143	418.777	158767.7	23	15	10
4	1	-0.004	899.932	449.08	140291.5	0	16	10
4	1	-0.004	899.93	389.278	140504.3	0	16	10
4	1	-0.004	899.928	448.48	140749.6	1	17	10
4	1	-0.005	899.925	470.426	141006.8	1	17	10
4	1	-0.004	899.923	453.866	141255	2	18	10
4	1	-0.004	899.92	442.829	141497.1	2	18	10
4	1	-0.004	899.918	426.326	141730.5	3	19	10
4	1	-0.004	899.916	431.928	141966.3	4	20	10
4	1	-0.004	899.914	436.655	142204.9	4	20	10

4	1	-0.003	899.912	378.529	142411.8	5	21	10
4	1	0.007	899.916	381.569	142620.5	5	21	10
4	1	0	899.915	295.324	142782	6	22	10
4	1	-0.002	899.914	225.873	142905.5	6	22	10
4	1	0.013	899.921	318.316	143075.9	7	23	10
4	1	0.007	899.925	275.393	143226.4	7	23	10
4	1	0.002	963.265	185.255	161706	8	0	11
4	1	1.231	963.938	123.012	161773.3	8	0	11
4	1	3.164	965.635	169.114	161864	9	1	11
4	1	14.601	973.617	812.145	162308	10	2	11
4	1	13.148	980.797	930.848	162816.3	10	2	11
4	1	7.221	984.745	765.868	163235	11	3	11
4	1	5.979	988.01	1021.725	163793	11	3	11
4	1	4.257	990.338	1151.372	164422.7	12	4	11
4	1	3.226	992.1	1197.912	165076.9	12	4	11
4	1	3.07	993.779	1272.739	165773	13	5	11
4	1	3.297	995.581	1317.47	166493.2	13	5	11
4	1	3.268	997.366	1258.815	167180.7	14	6	11
4	1	3.139	999.082	1281.092	167881	14	6	11
4	1	2.455	1000.422	1219.563	168547	15	7	11
4	1	1.73	1001.368	1174.615	169189.1	16	8	11
4	1	0.914	1001.868	950.093	169709.3	16	8	11
4	1	0.481	1002.131	817.433	170155.7	17	9	11
4	1	0.365	1002.33	903.671	170649.5	17	9	11
4	1	0.219	1002.45	883.218	171132.3	18	10	11
4	1	0.141	1002.527	915.561	171632.8	18	10	11
4	1	0.032	1002.545	814.983	172077.9	19	11	11
4	1	-0.007	1002.541	691.947	172456.1	19	11	11
4	1	-0.006	1002.538	561.271	172762.7	20	12	11
4	1	-0.003	1002.536	316.43	172935.5	20	12	11
4	1	-0.004	1002.534	406.105	173157.4	21	13	11
4	1	-0.005	1002.531	540.116	173452.5	22	14	11
4	1	-0.005	1002.528	524.209	173739.2	22	14	11
4	1	-0.005	1002.526	481.769	174002.3	23	15	11
4	1	-0.004	1002.524	358.843	174198.3	23	15	11
4	1	-0.004	963.141	448.839	159013	0	16	11
4	1	-0.004	963.138	434.124	159250.5	0	16	11
4	1	-0.004	963.136	387.258	159462.2	1	17	11
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4	1	-0.003	963.132	324.205	159844.1	2	18	11
4	1	-0.003	963.131	332.276	160025.8	2	18	11
4	1	-0.003	963.129	317.91	160199.5	3	19	11
4	1	-0.003	963.127	343.872	160387.4	4	20	11

4	1	0	963.127	300.355	160551.6	4	20	11
4	1	0.011	963.133	319.206	160726.2	5	21	11
4	1	0.028	963.148	332.392	160904.2	5	21	11
4	1	0.037	963.168	313.937	161072.4	6	22	11
4	1	0.051	963.196	319.57	161246.9	6	22	11
4	1	0.036	963.215	285.092	161399.6	7	23	11
4	1	0.088	963.264	374.541	161604.7	7	23	11
4	1	0	1002.501	338.275	176609.6	8	0	12
4	1	5.282	1005.39	729.053	177008.4	8	0	12
4	1	-0.002	1002.522	248.871	174331.5	0	16	12
4	1	-0.003	1002.521	276.792	174479.6	0	16	12
4	1	-0.003	1002.519	346.403	174669.1	1	17	12
4	1	-0.003	1002.517	339.745	174854.7	1	17	12
4	1	-0.004	1002.515	355	175048.7	2	18	12
4	1	-0.003	1002.514	322.733	175225.1	2	18	12
4	1	-0.003	1002.512	302.059	175387	3	19	12
4	1	-0.003	1002.51	289.974	175545.4	4	20	12
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4	1	-0.003	1002.508	262.049	175823.5	5	21	12
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4	1	-0.002	1002.505	248.064	176096.8	6	22	12
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4	1	-0.002	1002.503	183.325	176299.3	7	23	12
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4	2	0.046	36.867	255.75	43441.76	8	0	8
4	2	-0.003	39.471	254.832	51366.06	8	0	8
4	2	0.191	39.572	393.031	51572.29	8	0	8
4	2	0.523	37.153	402.586	43661.84	9	1	8
4	2	1.216	37.817	368.353	43863	9	1	8
4	2	1.827	40.551	463.184	51820.48	9	1	8
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4	2	4.273	42.887	542.407	52117	10	2	8
4	2	5.097	45.682	617.408	52455.54	10	2	8
4	2	0.485	39.54	542.031	44668.81	11	3	8
4	2	0.093	39.591	596.237	44994.75	11	3	8
4	2	3.969	47.853	684.993	52830.2	11	3	8
4	2	2.629	49.29	693.455	53209.29	11	3	8
4	2	-0.017	39.581	535.787	45287.65	12	4	8
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4	2	1.375	50.041	631.263	53554.02	12	4	8
4	2	1.433	50.825	627.309	53897.13	12	4	8
4	2	-0.015	39.568	538.896	45826.29	13	5	8

4	2	-0.018	39.558	567.147	46136.18	13	5	8
4	2	0.187	50.927	620.194	54235.82	13	5	8
4	2	0.704	51.311	647.343	54589.34	13	5	8
4	2	-0.018	39.549	543.63	46433.66	14	6	8
4	2	0.473	51.57	633.066	54935.77	14	6	8
4	2	0.373	51.774	664.862	55298.86	14	6	8
4	2	-0.019	39.538	532.897	46724.83	15	7	8
4	2	-0.019	39.528	504.752	47000.76	15	7	8
4	2	0.25	51.91	617.073	55636.02	15	7	8
4	2	-0.017	39.519	454.602	47249.78	16	8	8
4	2	-0.014	39.511	390.877	47463.67	16	8	8
4	2	0.003	51.912	491.025	55904.18	16	8	8
4	2	-0.004	51.91	381.554	56112.65	16	8	8
4	2	-0.003	39.509	122.361	47530.6	17	9	8
4	2	-0.002	39.508	147.559	47611.26	17	9	8
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4	2	-0.005	51.904	493.939	56632.83	17	9	8
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4	2	-0.003	39.506	256.766	47802.26	18	10	8
4	2	-0.005	51.902	493.853	56902.53	18	10	8
4	2	-0.005	51.899	475.78	57162.36	18	10	8
4	2	-0.002	39.506	184.767	47901.21	19	11	8
4	2	-0.003	39.504	297.804	48063.85	19	11	8
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4	2	-0.005	51.894	463.89	57678.38	19	11	8
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4	2	-0.004	51.892	420.237	57908	20	12	8
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4	2	-0.003	39.501	308.526	48402.38	21	13	8
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4	2	-0.004	39.493	355.933	49166	23	15	8
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4	2	-0.002	39.491	156.797	49406.42	0	16	8
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4	2	-0.003	39.485	285.555	50011.36	2	18	8
4	2	-0.003	39.483	272.965	50160.43	3	19	8
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4	2	-0.002	39.481	230.959	50428.35	4	20	8
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4	2	-0.002	39.478	249.605	50699.02	5	21	8
4	2	-0.002	39.477	230.589	50825.14	5	21	8
4	2	-0.002	39.475	221.52	50946.24	6	22	8
4	2	-0.002	39.475	173.088	51038.94	6	22	8
4	2	-0.002	39.474	176.775	51135.48	7	23	8
4	2	-0.002	39.473	175.773	51229.52	7	23	8
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4	2	0.202	51.965	434.744	62151.39	9	1	9
4	2	1.225	52.634	438.735	62390.99	9	1	9
4	2	2.397	53.943	446.006	62634.56	10	2	9
4	2	2.941	55.552	586.913	62955.57	10	2	9
4	2	2.695	57.026	718.481	63348.54	11	3	9
4	2	1.56	57.88	671.297	63715.89	11	3	9
4	2	1.356	58.621	641.214	64066.24	12	4	9
4	2	0.367	58.821	643.987	64418.29	12	4	9
4	2	0.69	59.199	670.602	64784.88	13	5	9
4	2	0.537	59.492	698.269	65166.41	13	5	9
4	2	0.318	59.666	599.285	65494.35	14	6	9
4	2	0.212	59.782	561.218	65800.99	15	7	9
4	2	0.233	59.909	630.666	66145.76	15	7	9
4	2	0.148	59.99	601.835	66474.76	16	8	9
4	2	0.051	60.018	536.308	66767.79	16	8	9
4	2	0.027	60.033	563.43	67075.64	17	9	9
4	2	0.015	60.041	573.33	67388.74	17	9	9
4	2	0.001	60.042	567.96	67698.91	18	10	9
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4	2	-0.005	60.036	513.086	68275.39	19	11	9
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4	2	-0.005	60.031	484.726	68805.09	20	12	9
4	2	-0.004	60.029	455.005	69053.57	21	13	9
4	2	-0.005	60.026	470.063	69310.41	21	13	9
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4	2	-0.003	60.02	308.221	69946.23	23	15	9
4	2	-0.003	60.018	345.14	70134.72	23	15	9
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4	2	-0.004	51.875	367.951	59580.65	0	16	9
4	2	-0.004	51.873	375.931	59785.95	1	17	9

4	2	-0.004	51.871	373.83	59990.21	1	17	9
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4	2	-0.003	51.868	323.054	60355.2	3	19	9
4	2	-0.003	51.866	309.538	60524.41	3	19	9
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4	2	-0.003	51.863	308.39	60868.38	4	20	9
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4	2	-0.003	51.858	259.884	61325.44	6	22	9
4	2	-0.003	51.857	267.541	61471.62	6	22	9
4	2	-0.002	51.855	252.287	61609.68	7	23	9
4	2	-0.002	51.854	248.626	61745.53	7	23	9
4	2	0.109	60.052	338.212	72862.14	8	0	10
4	2	0.873	60.529	484.64	73127.08	9	1	10
4	2	2.086	61.669	395.722	73343.3	9	1	10
4	2	2.627	63.104	377.55	73549.48	10	2	10
4	2	1.36	63.847	257.308	73690.08	10	2	10
4	2	1.535	64.686	355.985	73884.78	11	3	10
4	2	0.978	65.221	354.996	74078.84	11	3	10
4	2	0.703	65.605	428.469	74312.95	12	4	10
4	2	0.433	65.842	416.056	74540.16	12	4	10
4	2	0.351	66.034	461.489	74792.45	13	5	10
4	2	0.201	66.143	430.849	75027.73	13	5	10
4	2	0.101	66.199	408.721	75250.95	14	6	10
4	2	0.092	66.249	462.313	75503.42	15	7	10
4	2	0.085	66.296	486.351	75769.7	15	7	10
4	2	0.045	66.32	482.214	76033.04	16	8	10
4	2	0.013	66.327	499.252	76306.24	16	8	10
4	2	0.002	66.328	504.559	76581.93	17	9	10
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4	2	-0.003	66.319	338.48	77527.06	19	11	10
4	2	-0.002	66.318	234.885	77655.47	19	11	10
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4	2	-0.004	66.312	449.927	78304.73	21	13	10
4	2	-0.004	66.309	425.481	78537.56	22	14	10
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4	2	-0.004	66.305	406.332	78993	23	15	10
4	2	-0.004	66.303	363.018	79191.35	23	15	10
4	2	-0.003	60.016	324.307	70311.91	0	16	10
4	2	-0.004	60.014	399.076	70530.3	0	16	10

4	2	-0.004	60.012	444.459	70773.14	1	17	10
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4	2	-0.004	60.005	378.47	71410.01	3	19	10
4	2	-0.003	60.004	338.384	71595.27	3	19	10
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4	2	-0.003	60	316.071	71952.53	4	20	10
4	2	-0.003	59.999	279.696	72105.51	5	21	10
4	2	-0.003	59.997	274.316	72255.47	5	21	10
4	2	-0.002	59.996	183.694	72355.84	6	22	10
4	2	-0.002	59.995	203.779	72467.23	6	22	10
4	2	-0.002	59.994	208.858	72579.15	7	23	10
4	2	-0.002	59.993	178.86	72676.88	7	23	10
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4	2	0.806	66.928	491.753	81921.67	9	1	11
4	2	3.935	69.085	497.733	82194.59	9	1	11
4	2	5.589	72.138	495.237	82465.05	10	2	11
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4	2	3.52	76.764	459.992	82967.05	11	3	11
4	2	2.942	78.372	587.646	83288.3	11	3	11
4	2	1.87	79.394	596.595	83614.28	12	4	11
4	2	1.19	80.043	616.942	83951.2	12	4	11
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4	2	0.606	80.894	623.122	84674.28	13	5	11
4	2	0.425	81.127	631.414	85019.45	14	6	11
4	2	0.376	81.332	678.922	85390.22	15	7	11
4	2	0.257	81.473	663.73	85752.69	15	7	11
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4	2	-0.003	81.516	292.348	86216.69	16	8	11
4	2	-0.005	81.514	456.444	86466.09	17	9	11
4	2	-0.005	81.511	494.655	86736.36	17	9	11
4	2	-0.005	81.509	479.666	86998.45	18	10	11
4	2	-0.005	81.506	527.092	87286.59	18	10	11
4	2	-0.005	81.503	493.147	87556.17	19	11	11
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4	2	-0.004	66.299	358.103	79582.59	0	16	11
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4	2	-0.002	66.291	299.912	80427.48	3	19	11
4	2	-0.002	66.29	293.044	80587.68	4	20	11
4	2	0	66.29	248.283	80723.48	4	20	11
4	2	0.016	66.299	246.794	80858.39	5	21	11
4	2	0.048	66.325	281.683	81009.41	5	21	11
4	2	0.069	66.361	263.571	81150.41	6	22	11
4	2	0.054	66.391	236.145	81279.51	6	22	11
4	2	0.058	66.422	226.493	81400.87	7	23	11
4	2	0.043	66.445	184.862	81501.98	7	23	11
4	2	0.152	81.552	358.278	91779.45	8	0	12
4	2	-0.003	81.484	314.593	89511.78	0	16	12
4	2	-0.004	81.482	397.035	89724.2	0	16	12
4	2	-0.004	81.48	372.651	89928.12	1	17	12
4	2	-0.003	81.478	349.506	90119.09	1	17	12
4	2	-0.003	81.476	345.68	90308.05	2	18	12
4	2	-0.003	81.474	308.68	90476.8	3	19	12
4	2	-0.003	81.473	258.721	90615.36	3	19	12
4	2	-0.003	81.472	263.163	90759.08	4	20	12
4	2	-0.003	81.47	268.707	90902.84	4	20	12
4	2	-0.002	81.469	268.579	91049.73	5	21	12
4	2	-0.002	81.468	226.3	91171.05	5	21	12
4	2	-0.001	81.468	219.413	91288.56	6	22	12
4	2	0.004	81.47	211.039	91401.59	6	22	12
4	2	0.003	81.471	190.068	91503.27	7	23	12
4	2	-0.001	81.471	154.728	91587.77	7	23	12
4	3	0.05	261.341	90.842	32453.39	8	0	8
4	3	0.174	261.436	294.248	32614.25	8	0	8
4	3	-0.001	289.385	63.537	40024.88	8	0	8
4	3	0	289.385	36.149	40044.23	8	0	8
4	3	1.878	262.462	502.243	32888.66	9	1	8
4	3	5.991	265.734	507.284	33165.7	9	1	8
4	3	0.442	289.617	91.995	40092.48	9	1	8
4	3	4.78	292.178	412.974	40313.77	9	1	8
4	3	9.352	270.841	547.217	33464.54	10	2	8
4	3	9.08	275.805	551.34	33765.93	10	2	8
4	3	8.456	296.808	573.323	40627.66	10	2	8
4	3	9.418	301.969	668.121	40993.83	10	2	8
4	3	7.85	280.099	592.684	34090.1	11	3	8

4	3	5.893	283.324	614.599	34426.42	11	3	8
4	3	8.881	306.827	758.363	41408.61	11	3	8
4	3	6.855	310.571	794.076	41842.26	11	3	8
4	3	3.708	285.349	524.012	34712.59	12	4	8
4	3	2.371	286.645	467.866	34968.35	12	4	8
4	3	4.804	313.194	838.366	42300.1	12	4	8
4	3	3.29	314.994	866.676	42774.13	12	4	8
4	3	2.11	287.8	567.061	35278.66	13	5	8
4	3	2.472	316.344	908.711	43270.38	13	5	8
4	3	1.332	288.527	556.73	35583	14	6	8
4	3	0.785	288.957	548.334	35882.76	14	6	8
4	3	1.839	317.349	939.131	43783.77	14	6	8
4	3	1.25	318.032	913.046	44282.91	14	6	8
4	3	0.44	289.197	539.8	36177.7	15	7	8
4	3	0.238	289.327	545.493	36476.2	15	7	8
4	3	0.91	318.53	911.205	44780.52	15	7	8
4	3	0.69	318.907	880.775	45262.02	15	7	8
4	3	0.059	289.36	505.71	36753.08	16	8	8
4	3	0.095	289.412	562.735	37061.02	16	8	8
4	3	0.378	319.113	746.489	45669.68	16	8	8
4	3	0.271	319.261	682.675	46042.69	16	8	8
4	3	0	289.412	380.343	37269.15	17	9	8
4	3	-0.002	289.411	206.352	37379.55	17	9	8
4	3	0.276	319.412	715.644	46434.3	17	9	8
4	3	0.241	319.544	734.741	46835.55	17	9	8
4	3	-0.002	289.41	152.227	37462.68	18	10	8
4	3	-0.001	289.41	116.865	37526.6	18	10	8
4	3	0.162	319.632	738.104	47238.64	18	10	8
4	3	0.04	319.654	659.478	47598.79	18	10	8
4	3	-0.001	289.409	69.311	37563.7	19	11	8
4	3	-0.006	319.651	603.624	47928.77	19	11	8
4	3	-0.002	289.409	166.063	37654.39	20	12	8
4	3	-0.001	289.408	150.672	37736.76	20	12	8
4	3	-0.006	319.647	585.378	48249.1	20	12	8
4	3	-0.004	319.645	431.942	48485.11	20	12	8
4	3	-0.003	289.406	254.603	37876.08	21	13	8
4	3	-0.003	289.405	289.742	38034.31	21	13	8
4	3	-0.005	319.643	465.213	48739.68	21	13	8
4	3	-0.004	319.64	383.098	48949.32	21	13	8
4	3	-0.003	289.403	315.058	38206.54	22	14	8
4	3	-0.003	289.401	318.09	38380.25	22	14	8
4	3	-0.004	319.638	421.01	49179.36	22	14	8
4	3	-0.004	319.636	424.256	49411.17	22	14	8

4	3	-0.003	289.4	294.592	38541.13	23	15	8
4	3	-0.002	289.399	228.199	38666.01	23	15	8
4	3	-0.004	319.634	421.088	49641.25	23	15	8
4	3	-0.004	319.631	406.276	49863.12	23	15	8
4	3	-0.001	289.398	126.604	38735.25	0	16	8
4	3	-0.001	289.398	66.065	38770.64	0	16	8
4	3	-0.002	289.396	250.895	38907.86	1	17	8
4	3	-0.002	289.395	234.166	39035.94	2	18	8
4	3	-0.002	289.394	231.366	39162.42	2	18	8
4	3	-0.002	289.392	226.589	39286.16	3	19	8
4	3	-0.002	289.391	198.986	39394.94	3	19	8
4	3	-0.002	289.39	223.985	39517.38	4	20	8
4	3	-0.002	289.389	214.477	39634.51	4	20	8
4	3	-0.002	289.388	172.775	39729.11	5	21	8
4	3	-0.002	289.387	174.61	39824.61	5	21	8
4	3	-0.001	289.386	129.881	39895.54	6	22	8
4	3	-0.001	289.386	71.804	39934	6	22	8
4	3	-0.001	289.386	104.105	39990.85	7	23	8
4	3	-0.002	319.608	205.65	52258.26	8	0	9
4	3	-0.003	319.606	350.651	52449.95	8	0	9
4	3	1.184	320.254	739.413	52854.16	9	1	9
4	3	3.868	322.366	817.831	53300.78	9	1	9
4	3	4.584	324.872	803.87	53740.23	10	2	9
4	3	4.408	327.28	981.74	54276.64	10	2	9
4	3	4.105	329.526	1101.617	54879.47	11	3	9
4	3	2.849	331.084	1103.99	55483.29	11	3	9
4	3	1.976	332.164	1095.433	56082.13	12	4	9
4	3	1.474	332.971	1095.086	56681.07	12	4	9
4	3	1.063	333.551	1139.742	57303.5	13	5	9
4	3	0.849	334.015	1116.807	57914.02	14	6	9
4	3	0.671	334.382	1001.075	58461.83	14	6	9
4	3	0.587	334.703	965.818	58989.54	15	7	9
4	3	0.606	335.034	1045.745	59560.93	15	7	9
4	3	0.507	335.311	992.481	60103.48	16	8	9
4	3	0.359	335.508	893.588	60591.98	16	8	9
4	3	0.293	335.668	843.567	61052.66	17	9	9
4	3	0.22	335.788	805.287	61492.43	17	9	9
4	3	0.14	335.865	777.549	61917.06	18	10	9
4	3	0.043	335.888	710.142	62305.27	18	10	9
4	3	-0.006	335.885	655.556	62663.46	19	11	9
4	3	-0.006	335.882	573.278	62976.85	20	12	9
4	3	-0.005	335.879	515.194	63258.92	20	12	9
4	3	-0.005	335.876	547.142	63557.72	21	13	9

4	3	-0.006	335.873	574.23	63871.64	21	13	9
4	3	-0.005	335.87	495.675	64142.47	22	14	9
4	3	-0.004	335.868	395.711	64358.79	22	14	9
4	3	-0.003	335.867	309.731	64528.11	23	15	9
4	3	-0.003	335.865	304.788	64694.64	23	15	9
4	3	-0.004	319.629	416.075	50090.34	0	16	9
4	3	-0.004	319.627	376.567	50296.41	0	16	9
4	3	-0.004	319.625	368.264	50497.62	1	17	9
4	3	-0.003	319.623	342.803	50684.83	2	18	9
4	3	-0.003	319.622	330.114	50865.2	2	18	9
4	3	-0.003	319.62	315.997	51037.77	3	19	9
4	3	-0.003	319.618	294.243	51198.54	3	19	9
4	3	-0.003	319.617	281.821	51352.45	4	20	9
4	3	-0.003	319.615	261.897	51495.62	4	20	9
4	3	-0.003	319.614	257.801	51636.62	5	21	9
4	3	-0.002	319.613	242.619	51769.18	5	21	9
4	3	-0.002	319.611	222.238	51890.8	6	22	9
4	3	-0.002	319.61	247.646	52026.04	6	22	9
4	3	-0.002	319.609	219.012	52145.95	7	23	9
4	3	-0.003	335.842	309.798	67023.23	8	0	10
4	3	0.665	336.206	704.21	67408.78	8	0	10
4	3	6.045	339.511	955.206	67930.96	9	1	10
4	3	8.716	344.276	974.185	68463.52	9	1	10
4	3	6.881	348.034	880.419	68944.32	10	2	10
4	3	4.068	350.256	717.291	69336.24	10	2	10
4	3	3.021	351.908	884.836	69819.95	11	3	10
4	3	2.125	353.07	942.243	70335.3	11	3	10
4	3	1.551	353.917	990.09	70876	12	4	10
4	3	1.205	354.576	1038.949	71443.67	12	4	10
4	3	0.924	355.081	1056.103	72020.71	13	5	10
4	3	0.742	355.486	1024.061	72579.96	14	6	10
4	3	0.726	355.883	1026.112	73140.34	14	6	10
4	3	0.741	356.288	990.464	73681.79	15	7	10
4	3	0.663	356.651	944.189	74198.21	15	7	10
4	3	0.548	356.95	857.194	74666.34	16	8	10
4	3	0.411	357.175	770.46	75088.16	16	8	10
4	3	0.291	357.334	719.885	75481.3	17	9	10
4	3	0.202	357.444	660.91	75843.16	17	9	10
4	3	0.083	357.49	571.489	76155.25	18	10	10
4	3	0.007	357.494	523.452	76441.7	19	11	10
4	3	-0.004	357.492	401.005	76660.8	19	11	10
4	3	-0.003	357.49	296.807	76823.05	20	12	10
4	3	-0.003	357.488	322.783	76999.78	20	12	10

4	3	-0.003	357.486	348.657	77190.38	21	13	10
4	3	-0.003	357.485	331.649	77371.59	21	13	10
4	3	-0.003	357.483	326.711	77550.38	22	14	10
4	3	-0.003	357.481	320.928	77725.73	22	14	10
4	3	-0.003	357.479	301.988	77890.98	23	15	10
4	3	-0.003	357.478	299.49	78054.54	23	15	10
4	3	-0.003	335.863	330.271	64875.1	0	16	10
4	3	-0.004	335.861	403.558	65095.93	0	16	10
4	3	-0.004	335.859	375.794	65301.16	1	17	10
4	3	-0.004	335.857	379.822	65509.01	2	18	10
4	3	-0.004	335.855	355.309	65703.15	2	18	10
4	3	-0.004	335.853	357.298	65898.67	3	19	10
4	3	-0.003	335.851	300.005	66062.76	3	19	10
4	3	-0.003	335.85	290.213	66221.25	4	20	10
4	3	-0.003	335.848	278.211	66373.41	4	20	10
4	3	-0.003	335.847	259.041	66515.16	5	21	10
4	3	-0.002	335.846	235.78	66643.99	5	21	10
4	3	-0.002	335.845	180.739	66742.7	6	22	10
4	3	-0.001	335.844	148.648	66823.95	6	22	10
4	3	-0.001	335.844	55.965	66853.95	7	23	10
4	3	-0.002	357.465	180.274	79400.53	8	0	11
4	3	0.153	357.548	369.068	79602.39	8	0	11
4	3	3.685	359.521	554.098	79899.14	9	1	11
4	3	10.983	365.538	604.927	80230.51	9	1	11
4	3	13.719	373.03	683.736	80603.91	10	2	11
4	3	9.804	378.384	626.496	80946.04	10	2	11
4	3	5.98	381.653	602.527	81275.42	11	3	11
4	3	4.056	383.87	731.266	81675.18	11	3	11
4	3	2.54	385.258	752.419	82086.3	12	4	11
4	3	1.806	386.245	798.403	82522.53	13	5	11
4	3	1.536	387.085	832.961	82978.34	13	5	11
4	3	1.329	387.812	794.351	83412.37	14	6	11
4	3	1.063	388.393	769.724	83832.94	14	6	11
4	3	1.105	388.996	863.467	84304.48	15	7	11
4	3	1.136	389.616	882.718	84786.55	15	7	11
4	3	0.781	390.044	697.242	85168.67	16	8	11
4	3	0.355	390.238	498.547	85440.94	16	8	11
4	3	0.481	390.501	649.666	85796.09	17	9	11
4	3	0.383	390.71	654.468	86153.68	17	9	11
4	3	0.221	390.831	631.051	86498.3	18	10	11
4	3	0.113	390.893	579.094	86814.88	19	11	11
4	3	0.007	390.897	518.461	87098.3	19	11	11
4	3	-0.004	390.895	400.077	87316.78	20	12	11

4	3	-0.003	390.893	347.095	87506.34	20	12	11
4	3	-0.004	390.891	367.146	87707.04	21	13	11
4	3	-0.003	390.889	334.417	87889.66	21	13	11
4	3	-0.003	390.887	317.803	88063.3	22	14	11
4	3	-0.003	390.886	332.141	88244.88	22	14	11
4	3	-0.002	390.884	218.771	88364.35	23	15	11
4	3	-0.001	390.884	96.919	88417.28	23	15	11
4	3	-0.003	357.476	287.393	78211.88	0	16	11
4	3	-0.002	357.475	249.654	78348.22	1	17	11
4	3	-0.002	357.474	229.768	78473.76	1	17	11
4	3	-0.002	357.472	226.619	78597.64	2	18	11
4	3	-0.002	357.471	201.845	78708.15	2	18	11
4	3	-0.002	357.47	178.594	78805.78	3	19	11
4	3	-0.002	357.469	170.061	78898.66	3	19	11
4	3	-0.002	357.469	159.252	78985.67	4	20	11
4	3	-0.001	357.468	151.842	79068.77	4	20	11
4	3	-0.001	357.467	149.098	79150.23	5	21	11
4	3	0	357.467	33.515	79168.2	5	21	11
4	3	-0.001	357.467	52.106	79196.08	6	22	11
4	3	-0.001	357.466	144.983	79275.34	7	23	11
4	3	0	357.466	49.546	79301.88	7	23	11
4	3	-0.001	390.876	128.26	89257.87	8	0	12
4	3	0.126	390.943	384.33	89463.7	8	0	12
4	3	-0.001	390.884	72.809	88456.27	0	16	12
4	3	-0.001	390.883	51.082	88483.62	1	17	12
4	3	-0.002	390.882	219.967	88603.93	1	17	12
4	3	-0.002	390.881	194.385	88710.2	2	18	12
4	3	-0.002	390.88	166.412	88801.12	2	18	12
4	3	-0.001	390.88	127.163	88870.67	3	19	12
4	3	-0.001	390.879	61.447	88903.56	3	19	12
4	3	-0.001	390.878	142.235	88981.24	4	20	12
4	3	-0.001	390.878	63.928	89015.48	4	20	12
4	3	-0.001	390.877	106.753	89073.87	5	21	12
4	3	-0.001	390.877	51.059	89101.21	5	21	12
4	3	-0.001	390.877	54.863	89130.59	6	22	12
4	3	0	390.877	34.836	89149.25	6	22	12
4	3	-0.001	390.876	72.094	89187.82	7	23	12
4	4	0.073	725.094	327.228	79305.8	8	0	8
4	4	1.944	726.157	674.352	79674.26	8	0	8
4	4	-0.002	859.753	225.982	91187.27	8	0	8
4	4	0.335	859.933	188.208	91288.07	8	0	8
4	4	10.645	731.97	488.15	79940.84	9	1	8
4	4	22.903	744.484	568.254	80251.33	9	1	8

4	4	11.412	866.051	358.602	91480.32	9	1	8
4	4	33.274	883.862	544.769	91771.92	9	1	8
4	4	31.224	761.536	663.985	80613.94	10	2	8
4	4	30.061	777.978	728.85	81012.58	10	2	8
4	4	55.156	914.091	702.324	92156.84	10	2	8
4	4	54.444	943.899	737.621	92560.68	10	2	8
4	4	23.441	790.792	765.08	81430.82	11	3	8
4	4	17.843	800.551	826.293	81882.76	11	3	8
4	4	46.111	969.119	758.582	92975.58	11	3	8
4	4	36.19	988.883	801.996	93413.55	11	3	8
4	4	9.81	805.908	572.254	82195.27	12	4	8
4	4	30.596	1005.609	880.807	93895.06	12	4	8
4	4	13.161	813.106	769.262	82616.02	13	5	8
4	4	16.464	822.116	852.81	83082.7	13	5	8
4	4	25.38	1019.476	901.607	94387.69	13	5	8
4	4	23.166	1032.134	952.544	94908.15	13	5	8
4	4	15.019	830.33	808.606	83524.96	14	6	8
4	4	14.304	838.142	894.502	84013.46	14	6	8
4	4	20.328	1043.252	983.001	95445.8	14	6	8
4	4	17.163	1052.625	978.789	95980.33	14	6	8
4	4	11.491	844.423	896.621	84503.62	15	7	8
4	4	8.849	849.27	864.748	84977.3	15	7	8
4	4	13.646	1060.077	965.254	96507.46	15	7	8
4	4	10.441	1065.785	936.26	97019.28	15	7	8
4	4	6.112	852.614	769.676	85398.27	16	8	8
4	4	4.641	855.153	783.554	85827.05	16	8	8
4	4	6.539	1069.356	728.795	97417.28	16	8	8
4	4	5.455	1072.338	801.422	97855.39	16	8	8
4	4	3.32	856.969	737.941	86230.66	17	9	8
4	4	2.373	858.238	703.794	86607.2	17	9	8
4	4	4.168	1074.618	786.585	98285.61	17	9	8
4	4	3.186	1076.358	760.771	98701.08	17	9	8
4	4	1.477	859.045	608.557	86939.53	18	10	8
4	4	2.263	1077.594	728.921	99099.15	18	10	8
4	4	0.508	859.317	349.66	87126.98	19	11	8
4	4	0.535	859.609	501.918	87401.09	19	11	8
4	4	1.437	1078.378	642.179	99449.85	19	11	8
4	4	0.844	1078.84	581.703	99768.01	19	11	8
4	4	0.218	859.728	415.791	87628.16	20	12	8
4	4	0.085	859.775	414.873	87855.07	20	12	8
4	4	0.359	1079.037	473.602	100027.2	20	12	8
4	4	0.131	1079.109	426.226	100260.1	20	12	8
4	4	0.017	859.784	440.784	88096.27	21	13	8

4	4	-0.005	859.781	460.583	88347.8	21	13	8
4	4	-0.004	1079.107	370.696	100462.9	21	13	8
4	4	-0.004	1079.105	385.357	100673.7	21	13	8
4	4	-0.004	859.779	392.069	88562.02	22	14	8
4	4	-0.004	859.777	365.952	88761.88	22	14	8
4	4	-0.004	1079.102	430.598	100909.1	22	14	8
4	4	-0.004	1079.1	434.374	101146.3	22	14	8
4	4	-0.003	859.776	334.106	88944.43	23	15	8
4	4	-0.002	859.775	152.834	89028.06	23	15	8
4	4	-0.004	1079.098	408.373	101369.4	23	15	8
4	4	-0.004	1079.096	397.254	101586.4	23	15	8
4	4	-0.002	859.774	210.161	89140.62	0	16	8
4	4	-0.003	859.772	279.791	89293.57	1	17	8
4	4	-0.003	859.771	273.461	89443.29	1	17	8
4	4	-0.003	859.769	306.412	89610.8	2	18	8
4	4	-0.003	859.767	308.18	89779.18	2	18	8
4	4	-0.003	859.766	300.357	89943.21	3	19	8
4	4	-0.003	859.764	292.533	90103.29	3	19	8
4	4	-0.003	859.763	273.41	90252.6	4	20	8
4	4	-0.003	859.761	265.525	90397.68	4	20	8
4	4	-0.003	859.76	269.974	90545.41	5	21	8
4	4	-0.003	859.758	283.064	90700.23	5	21	8
4	4	-0.002	859.757	189.721	90801.73	6	22	8
4	4	-0.002	859.756	239.661	90932.75	7	23	8
4	4	-0.002	859.755	249.526	91066.25	7	23	8
4	4	-0.002	1079.068	248.076	104384.7	8	0	9
4	4	0.418	1079.296	637.732	104733.1	8	0	9
4	4	9.056	1084.247	695.892	105113.6	9	1	9
4	4	16.879	1093.465	711.446	105502.1	9	1	9
4	4	20.74	1104.809	845.755	105964.7	10	2	9
4	4	17.186	1114.204	902.79	106458.2	10	2	9
4	4	13.128	1121.384	986.59	106997.8	11	3	9
4	4	10.036	1126.87	980.718	107533.9	11	3	9
4	4	6.989	1130.691	893.167	108022.2	12	4	9
4	4	6.703	1134.358	1012.666	108576.1	13	5	9
4	4	5.675	1137.457	1034.166	109140.8	13	5	9
4	4	4.42	1139.874	984.455	109679.3	14	6	9
4	4	2.956	1141.492	810.621	110122.9	14	6	9
4	4	2.936	1143.096	982.51	110659.7	15	7	9
4	4	2.533	1144.48	1001.602	111206.7	15	7	9
4	4	1.944	1145.542	894.779	111695.8	16	8	9
4	4	1.755	1146.502	920.198	112198.9	16	8	9
4	4	1.454	1147.296	879.769	112679.3	17	9	9

4	4	1.17	1147.935	840.513	113138.3	17	9	9
4	4	0.803	1148.373	757.76	113552.2	18	10	9
4	4	0.571	1148.685	754.436	113964.8	19	11	9
4	4	0.383	1148.895	729.453	114363.6	19	11	9
4	4	0.144	1148.974	618.228	114701.5	20	12	9
4	4	0.016	1148.982	537.9	114995.7	20	12	9
4	4	-0.005	1148.98	574.407	115309.4	21	13	9
4	4	-0.006	1148.977	559.944	115615.7	21	13	9
4	4	-0.005	1148.974	541.101	115911.3	22	14	9
4	4	-0.003	1148.972	276.495	116062.4	22	14	9
4	4	-0.004	1148.97	394.104	116277.8	23	15	9
4	4	-0.004	1079.094	362.567	101784.5	0	16	9
4	4	-0.004	1079.092	376.588	101990.4	1	17	9
4	4	-0.004	1079.09	358.621	102186.5	1	17	9
4	4	-0.004	1079.088	362.189	102384.3	2	18	9
4	4	-0.003	1079.086	346.678	102573.6	2	18	9
4	4	-0.004	1079.084	355.873	102768	3	19	9
4	4	-0.003	1079.082	325.539	102945.9	3	19	9
4	4	-0.003	1079.08	329.721	103126	4	20	9
4	4	-0.003	1079.078	347.377	103315.8	4	20	9
4	4	-0.003	1079.077	342.211	103503	5	21	9
4	4	-0.003	1079.075	336.568	103686.9	5	21	9
4	4	-0.003	1079.073	339.05	103872.5	6	22	9
4	4	-0.003	1079.071	336.435	104056.2	7	23	9
4	4	-0.003	1079.069	352.488	104249.2	7	23	9
4	4	0.252	1149.081	739.683	119957.4	8	0	10
4	4	4.644	1151.621	538.406	120251.9	8	0	10
4	4	23.028	1164.21	781.605	120679.2	9	1	10
4	4	25.8	1178.314	854.087	121146.1	9	1	10
4	4	22.459	1190.579	903.02	121639.2	10	2	10
4	4	15.596	1199.105	885.744	122123.4	10	2	10
4	4	11.758	1205.53	966.502	122651.5	11	3	10
4	4	8.948	1210.426	1014.536	123206.7	12	4	10
4	4	7.04	1214.271	1061.699	123786.5	12	4	10
4	4	6.265	1217.694	1194.009	124438.9	13	5	10
4	4	5.34	1220.61	1196.454	125092.3	13	5	10
4	4	4.293	1222.954	1105.34	125695.9	14	6	10
4	4	3.735	1224.995	1134.986	126316.1	14	6	10
4	4	2.798	1226.526	1024.762	126876.5	15	7	10
4	4	2.028	1227.634	912.226	127375.2	15	7	10
4	4	1.614	1228.516	877.865	127854.6	16	8	10
4	4	1.24	1229.195	828.276	128308.1	16	8	10
4	4	0.877	1229.673	770.676	128729	17	9	10

4	4	0.586	1229.994	695.149	129109.4	18	10	10
4	4	0.39	1230.207	651.551	129465.4	18	10	10
4	4	0.246	1230.341	603.805	129795.8	19	11	10
4	4	-0.003	1230.34	308.502	129964.4	19	11	10
4	4	0.036	1230.359	478.487	130226.1	20	12	10
4	4	0.025	1230.373	526.4	130514	20	12	10
4	4	0.004	1230.375	539.642	130809	21	13	10
4	4	-0.002	1230.374	537.84	131103.2	21	13	10
4	4	-0.005	1230.371	493.382	131372.9	22	14	10
4	4	-0.005	1230.369	480.753	131635.6	22	14	10
4	4	-0.005	1230.366	466.04	131890.6	23	15	10
4	4	-0.004	1148.968	363.018	116476.3	0	16	10
4	4	-0.004	1148.966	455.599	116725.2	0	16	10
4	4	-0.004	1148.963	454.018	116973.7	1	17	10
4	4	-0.004	1148.961	440.571	117214.3	1	17	10
4	4	-0.004	1148.959	433.888	117451.8	2	18	10
4	4	-0.004	1148.956	435.184	117689.5	2	18	10
4	4	-0.004	1148.954	442.584	117931.8	3	19	10
4	4	-0.004	1148.952	430.349	118166.9	3	19	10
4	4	-0.004	1148.949	415.025	118393.6	4	20	10
4	4	-0.004	1148.947	399.588	118612.3	4	20	10
4	4	-0.004	1148.945	398.453	118830.2	5	21	10
4	4	-0.004	1148.943	398.489	119048	6	22	10
4	4	-0.003	1148.941	277.54	119199.6	6	22	10
4	4	-0.001	1148.941	324.564	119373.4	7	23	10
4	4	0.005	1148.943	328.09	119552.9	7	23	10
4	4	0.201	1230.764	455.432	135387.4	8	0	11
4	4	2.139	1231.933	695.571	135767.6	8	0	11
4	4	26.279	1246.029	703.626	136145.1	9	1	11
4	4	37.31	1266.436	775.299	136569.1	9	1	11
4	4	32.162	1284	821.516	137017.8	10	2	11
4	4	19.469	1294.637	710.343	137405.9	10	2	11
4	4	14.787	1302.717	878.441	137885.8	11	3	11
4	4	10.389	1308.396	969.78	138416	12	4	11
4	4	7.276	1312.374	992.151	138958.4	12	4	11
4	4	5.785	1315.535	1083.36	139550.3	13	5	11
4	4	4.662	1318.086	1107.416	140156.3	13	5	11
4	4	3.75	1320.134	1041.832	140725.3	14	6	11
4	4	3.237	1321.903	1049.402	141298.6	14	6	11
4	4	2.712	1323.384	1028.082	141860.1	15	7	11
4	4	2.225	1324.6	977.115	142394	15	7	11
4	4	1.2	1325.257	645.7	142747.7	16	8	11
4	4	1.338	1325.988	773.153	143169.9	16	8	11

4	4	1.127	1326.604	741.928	143575.5	17	9	11
4	4	1.103	1327.207	830.521	144029.5	18	10	11
4	4	0.766	1327.626	776.031	144453.3	18	10	11
4	4	0.51	1327.904	741.596	144858.5	19	11	11
4	4	0.314	1328.076	716.927	145250.4	19	11	11
4	4	0.079	1328.119	554.656	145553.3	20	12	11
4	4	0.044	1328.143	602.37	145882.3	20	12	11
4	4	0.004	1328.146	624.205	146223.5	21	13	11
4	4	-0.005	1328.143	577.988	146539.2	21	13	11
4	4	-0.006	1328.14	577.417	146854.8	22	14	11
4	4	-0.005	1328.137	534.244	147146.8	22	14	11
4	4	-0.003	1328.135	343.001	147334.1	23	15	11
4	4	-0.005	1230.364	470.58	132147.7	0	16	11
4	4	-0.004	1230.361	444.08	132390.7	0	16	11
4	4	-0.004	1230.359	428.551	132624.9	1	17	11
4	4	-0.004	1230.357	408.798	132848.4	1	17	11
4	4	-0.004	1230.354	401.906	133068.1	2	18	11
4	4	-0.004	1230.352	378.592	133275.1	2	18	11
4	4	-0.004	1230.35	408.502	133498.4	3	19	11
4	4	-0.003	1230.348	387.281	133710	3	19	11
4	4	0.001	1230.349	389.016	133922.6	4	20	11
4	4	0.031	1230.366	382.655	134132	4	20	11
4	4	0.069	1230.402	386.196	134338.7	5	21	11
4	4	0.09	1230.45	368.925	134536.4	6	22	11
4	4	0.111	1230.511	376.382	134741.9	6	22	11
4	4	0.137	1230.584	376.27	134943.5	7	23	11
4	4	0.127	1230.654	355.88	135138.3	7	23	11
4	4	0.021	1328.135	338.612	150423.6	8	0	12
4	4	3.907	1330.272	896.403	150914.2	8	0	12
4	4	-0.003	1328.134	321.074	147505.9	0	16	12
4	4	-0.004	1328.131	397.979	147719.1	0	16	12
4	4	-0.005	1328.129	463.786	147972.5	1	17	12
4	4	-0.004	1328.127	394.956	148188.4	1	17	12
4	4	-0.004	1328.125	407.325	148411	2	18	12
4	4	-0.004	1328.123	408.895	148634.5	2	18	12
4	4	-0.004	1328.12	390.062	148843.6	3	19	12
4	4	-0.004	1328.119	369.26	149045.2	3	19	12
4	4	-0.003	1328.117	354.605	149235	4	20	12
4	4	-0.003	1328.115	346.982	149424.8	4	20	12
4	4	-0.003	1328.113	351.708	149613.1	5	21	12
4	4	0.002	1328.114	337.437	149793.8	5	21	12
4	4	-0.001	1328.114	257.954	149932	6	22	12
4	4	0.007	1328.118	261.949	150072.3	7	23	12

4	4	0.01	1328.123	311.716	150242.5	7	23	12

[1] Negative numbers were set to 0. [2] Value at 0 h was set to 0 and 24 h accumulation calculated from difference

VFA Enrichment Data

Period	Fermenter	Hour after feeding	Sample Day	Acetate	Propionate	Butyrate	Isovalerate	Valerate
1	1	2	8	1.121711	1.099396	1.312641	1.094468	1.096839
1	1	4	8	1.101151	1.093433	1.23234	.	.
1	1	16	8	1.980291	1.149586	.	1.349607	1.241716
1	1	22	8	1.153948	1.111171	1.404308	1.110238	1.109583
1	1	26	8	1.124112	1.096742	1.377951	1.104448	1.09853
1	1	30	8	1.114398	1.100189	1.276933	1.096337	1.096741
1	1	36	8	1.104515	1.148381	1.146195	1.130251	1.150196
1	1	0	10	1.11942	6.203589	1.185766	1.413202	1.372316
1	1	2	10	1.117584	5.987465	1.166726	1.739768	1.695756
1	1	4	10	1.119309	5.355293	1.147308	2.168731	1.839346
1	1	6	10	1.12507	5.090089	1.147359	2.461481	1.949467
1	1	8	10	1.13177	5.055051	1.143446	2.564039	2.074723
1	1	12	10	1.14238	5.285045	1.14846	2.654235	2.270947
1	1	16	10	1.153687	6.233621	1.157329	2.69092	2.427766
1	1	22	10	1.166727	7.74953	1.165777	2.661016	2.411123
1	1	26	10	1.145176	5.941517	1.166986	2.750532	2.458871
1	1	30	10	1.13822	3.642634	1.132782	2.692287	2.258745
1	1	36	10	1.14131	.	1.131391	2.407192	2.234445
1	1	0	12	1.962543	2.420072	1.199653	1.928483	1.950382
1	1	2	12	1.933314	1.972695	1.362333	1.904616	1.809659
1	1	4	12	1.810126	1.719376	1.428037	1.820821	1.640143
1	1	6	12	1.682973	1.542366	1.409286	1.713878	1.458591
1	1	8	12	1.653382	1.471074	1.419639	1.68979	1.47217
1	1	12	12	1.667942	1.390706	1.434109	1.599805	1.443587
1	1	16	12	1.893796	1.377347	1.444083	1.561733	1.42678
1	1	22	12	2.255945	1.368426	1.514633	1.519238	1.405647
1	2	2	8	1.954987	1.121369	1.278635	1.204758	1.134345
1	2	4	8	1.88119	1.126042	1.278189	.	.
1	2	16	8	1.153432	5.476177	.	2.484586	2.267465
1	2	22	8	2.559614	1.150581	1.465924	1.364519	1.251191
1	2	26	8	2.261384	1.127617	1.500146	1.387458	1.229563
1	2	30	8	1.66986	1.145657	1.386909	1.375963	1.190913
1	2	36	8	1.426263	1.160039	1.385177	1.317567	1.255573
1	2	0	10	1.33905	1.351506	1.543286	1.385709	1.45287
1	2	2	10	1.245606	1.172757	1.48974	1.253747	1.241338

1	2	4	10	1.199801	1.133317	1.408657	1.203053	1.173908
1	2	6	10	1.160677	1.190455	1.35462	1.183946	1.176436
1	2	8	10	1.16019	1.111726	1.340417	1.1612	1.142259
1	2	12	10	1.151896	1.107892	1.314515	1.142294	1.134686
1	2	16	10	1.150984	1.106018	1.356432	1.133831	1.130252
1	2	22	10	1.152778	1.105906	1.437489	1.131885	1.1313
1	2	26	10	1.143365	1.208063	1.345465	1.2023	1.243764
1	2	30	10	1.123865	1.104394	1.191484	1.125623	1.137716
1	2	36	10	1.121502	1.126258	1.158283	1.173196	1.205634
1	2	0	12	1.163059	5.781402	1.170148	1.459147	1.468625
1	2	2	12	1.15656	5.109619	1.194637	1.812996	1.64997
1	2	6	12	1.135605	4.605863	1.077551	2.239554	1.763248
1	2	8	12	1.14168	4.616921	1.080644	2.374726	1.893135
1	2	12	12	1.164453	4.983445	1.067383	2.476697	2.064393
1	2	16	12	1.17906	5.874388	1.069237	2.500888	2.121473
1	2	22	12	1.189379	7.502844	1.14819	2.55813	2.333637
1	2	26	12	1.158612	6.008899	1.180137	2.809611	2.318936
1	3	2	8	1.152448	1.230872	1.568862	1.091766	1.094505
1	3	4	8	1.103656	1.094478	1.324092	.	.
1	3	12	8	1.13118	1.098966	1.418996	1.108131	1.100929
1	3	16	8	2.049609	1.164854	.	1.341615	1.265606
1	3	22	8	1.163987	1.104033	1.458093	1.118739	1.110451
1	3	26	8	1.128684	1.096959	1.4923	1.11228	1.105425
1	3	30	8	1.140345	1.088595	1.299931	1.106868	1.104753
1	3	36	8	1.110164	1.104264	1.163004	1.106254	1.115171
1	3	0	10	1.143219	5.183257	1.177045	1.562033	1.334669
1	3	2	10	1.131979	4.622707	1.161189	1.984632	1.565802
1	3	4	10	1.133765	4.156211	1.155225	2.214951	1.657977
1	3	6	10	1.139983	4.121744	1.153526	2.322351	1.75702
1	3	8	10	1.1438	4.215456	1.155266	2.375273	1.824479
1	3	12	10	1.151083	4.688805	1.158314	2.509512	1.943441
1	3	16	10	1.162768	5.360442	1.168547	2.606918	2.040952
1	3	22	10	1.180756	5.17617	1.163707	2.650755	2.101955
1	3	26	10	1.164576	5.601023	1.178933	2.690957	2.006134
1	3	30	10	1.146929	3.25029	1.150568	2.549909	1.9821
1	3	36	10	1.140174	2.53591	1.142244	2.155674	1.855172
1	3	0	12	1.995404	2.052723	1.223834	1.738943	1.700008
1	3	6	12	1.692847	1.417066	1.35528	1.59229	1.428802
1	3	8	12	1.713268	1.396047	1.363847	1.568272	1.43237
1	3	12	12	1.765476	1.418528	1.425895	1.565516	1.47159
1	3	16	12	1.966997	1.335626	1.448734	1.503231	1.407165
1	3	22	12	2.243134	1.443814	1.515003	1.525657	1.451736
1	4	2	8	1.116454	1.113187	1.317478	1.091212	1.091071

1	4	4	8	1.113823	1.090926	1.272961	.	.
1	4	12	8	1.113916	1.096954	1.329267	1.10341	1.096749
1	4	16	8	1.09788	1.094852	.	1.102963	1.098052
1	4	26	8	1.118803	1.120622	1.322667	1.138259	1.136163
1	4	30	8	1.575746	1.158523	1.482583	1.294616	1.27206
1	4	36	8	1.112602	1.099412	1.167493	1.109425	1.111273
1	4	0	10	2.321845	1.12289	1.26673	1.171955	1.178919
1	4	2	10	2.043589	1.122273	1.436052	1.205616	1.190877
1	4	4	10	1.884809	1.127294	1.430182	1.232591	1.204364
1	4	6	10	1.889881	1.21425	1.474929	1.251985	1.226339
1	4	8	10	1.919424	1.161555	1.44753	1.264233	1.242792
1	4	12	10	2.035097	1.156006	1.474326	1.277897	1.270303
1	4	16	10	2.244807	1.163507	1.508415	1.283073	1.284411
1	4	22	10	2.691199	1.176596	1.569632	1.289236	1.322243
1	4	26	10	2.20711	1.161788	1.64926	1.287941	1.306415
1	4	30	10	1.605561	1.15323	1.492336	1.281847	1.277518
1	4	36	10	1.431766	1.147152	1.409038	1.246141	1.260639
1	4	0	12	1.325571	6.538939	1.31251	1.39156	1.477932
1	4	6	12	1.200719	5.182144	1.156802	1.79253	1.747105
1	4	8	12	1.191064	4.877076	1.090448	1.985272	1.940356
1	4	12	12	1.203991	5.120891	1.124666	2.08144	2.143738
1	4	16	12	1.205114	6.197165	1.106915	2.076093	2.151972
1	4	22	12	1.214383	8.401107	1.139559	2.055469	2.398779
1	4	26	12	1.189961	6.240469	1.208991	2.229414	2.412259
1	4	30	12	1.168708	4.223612	1.166529	2.323303	2.402778
1	4	36	12	1.162554	2.995729	1.151248	2.069256	2.302509
2	1	0	8	1.106174	1.09061	1.106734	1.101372	1.104685
2	1	2	8	1.111517	1.106031	1.356695	1.102332	1.105686
2	1	4	8	1.106295	1.090717	1.321528	1.093972	1.095094
2	1	6	8	1.107223	1.09699	1.237326	1.094976	1.094056
2	1	8	8	1.108431	1.091165	1.320535	1.09346	1.094837
2	1	12	8	1.125191	1.095092	1.28956	1.106581	1.10464
2	1	16	8	1.117044	1.091412	1.396123	1.096308	1.097725
2	1	22	8	1.113175	1.091082	1.419043	1.103144	1.100262
2	1	26	8	1.121569	1.103739	1.37999	1.103156	1.105314
2	1	30	8	1.119568	1.097584	1.222457	1.095199	1.098356
2	1	36	8	1.121199	1.095108	1.173761	1.096345	1.098327
2	1	0	10	1.131737	5.526145	1.078585	1.341861	1.333056
2	1	2	10	1.124838	5.243333	.	1.604234	1.64438
2	1	4	10	1.128411	4.85037	1.159177	1.859243	1.66839
2	1	6	10	1.140534	4.77001	1.141709	2.034722	1.809499
2	1	8	10	1.142365	4.612865	1.134872	2.164818	1.935079
2	1	12	10	1.165783	4.734225	1.150224	2.20006	2.003852

2	1	16	10	1.164131	5.185056	1.163266	2.31177	2.18157
2	1	22	10	1.200438	6.340691	1.190305	2.337591	2.290891
2	1	26	10	1.159897	5.297535	1.160229	2.49277	2.351712
2	1	30	10	1.14836	3.761965	1.14473	2.54609	2.204866
2	1	36	10	1.147082	2.719836	1.131795	2.213098	2.13207
2	1	0	12	2.141747	2.05449	1.217257	1.739405	1.835064
2	1	6	12	2.082949	1.467938	.	1.491875	1.51113
2	1	22	12	2.434194	1.391581	1.480645	1.486998	1.49247
2	2	0	8	1.999704	1.106613	1.181407	1.151781	1.118963
2	2	2	8	1.98378	1.130166	1.296219	1.177941	1.13675
2	2	4	8	1.794197	1.11745	1.320577	1.22639	1.142079
2	2	6	8	1.755349	1.131263	1.35291	1.256479	1.154659
2	2	8	8	1.739403	1.129363	1.352423	1.281488	1.175654
2	2	16	8	2.005177	1.168056	1.400184	1.311082	1.20603
2	2	22	8	2.305169	1.168609	1.442892	1.312631	1.232376
2	2	26	8	2.101616	1.158234	1.512224	1.343375	1.20685
2	2	30	8	1.606238	1.15364	1.428935	1.353133	1.200225
2	2	36	8	1.392349	1.139382	1.350373	1.304112	1.197594
2	2	0	10	1.320976	6.339337	1.35235	1.650624	1.547096
2	2	2	10	1.234617	1.227397	.	1.237833	1.221317
2	2	4	10	1.211225	5.008778	1.254416	2.231477	1.770103
2	2	6	10	1.20027	4.915593	1.207409	2.385179	1.919471
2	2	8	10	1.193884	4.987526	1.192075	2.420252	2.012442
2	2	16	10	1.208324	5.968288	1.201706	2.615023	2.320206
2	2	22	10	1.249611	7.923204	1.148445	2.58462	2.336252
2	2	26	10	1.187718	5.869212	1.187518	2.787817	2.3164
2	2	30	10	1.16949	4.127272	1.155199	2.779534	2.262977
2	2	36	10	1.164485	2.942723	1.135301	2.414525	2.199728
2	2	0	12	1.186367	2.2256	1.337299	1.831203	1.897045
2	2	8	12	1.191567	1.473709	.	1.441835	1.457385
2	2	16	12	1.132608	1.311432	.	1.318594	1.344847
2	2	22	12	1.164048	1.277958	1.438072	1.270228	1.313996
2	3	0	8	1.822685	1.111388	1.180046	1.151153	1.140236
2	3	2	8	1.887621	1.119599	1.241581	1.169717	1.12397
2	3	4	8	1.72546	1.101704	1.300806	1.213905	1.13812
2	3	6	8	1.673984	1.111682	1.297745	1.23278	1.15093
2	3	8	8	1.678533	1.117043	1.312573	1.250729	1.166771
2	3	12	8	1.702498	1.127541	1.329787	1.262023	1.169175
2	3	22	8	2.231351	1.19164	1.438521	1.276135	1.272892
2	3	26	8	1.979332	1.146687	1.479244	1.277001	1.187665
2	3	30	8	1.571956	1.145433	1.368238	1.302665	1.204505
2	3	36	8	1.414147	1.152065	1.365554	1.27593	1.232507
2	3	0	10	1.295635	1.27122	1.471134	1.231788	1.226658

2	3	2	10	1.24302	5.523119	1.261841	1.94435	1.596256
2	3	4	10	1.201269	1.179447	1.375844	1.226041	1.203222
2	3	6	10	1.184529	1.16242	1.332033	1.219291	1.202462
2	3	8	10	1.172731	1.159858	1.304172	1.201677	1.249534
2	3	16	10	1.153134	1.217433	1.310491	1.225416	1.274628
2	3	22	10	1.152116	1.19164	1.363424	1.276135	1.272892
2	3	26	10	1.141887	1.106523	1.369486	1.133338	1.130687
2	3	30	10	1.130458	1.088001	1.223346	1.118647	1.117053
2	3	36	10	1.120855	1.099938	1.161072	1.110271	1.11131
2	3	0	12	1.132506	6.434209	1.163379	1.489033	1.472447
2	3	6	12	1.169035	5.007484	.	1.844781	2.103809
2	3	8	12	1.135783	5.085548	.	2.496251	2.150655
2	3	22	12	1.181703	8.075836	1.121299	2.543882	2.434958
2	4	0	8	1.104695	1.117732	1.114221	1.114271	1.135262
2	4	2	8	1.10492	1.093359	1.375092	1.092538	1.09525
2	4	4	8	1.105164	1.094011	1.339618	1.091941	1.094294
2	4	6	8	1.106464	1.097321	1.298713	1.092117	1.095107
2	4	8	8	1.106789	1.141478	1.357169	1.09734	1.098554
2	4	12	8	1.149935	1.125369	1.318135	1.121366	1.131298
2	4	16	8	1.108327	1.095079	1.392753	1.094946	1.097931
2	4	22	8	1.129131	1.180667	1.430545	1.142847	1.184965
2	4	26	8	1.132537	1.098229	1.365727	1.107101	1.111883
2	4	30	8	1.116579	1.10136	1.257171	1.096848	1.100919
2	4	36	8	1.108538	1.096312	1.156011	1.094387	1.097272
2	4	0	10	1.978924	1.138822	1.251053	1.174934	1.150483
2	4	2	10	1.989642	1.18837	.	1.251281	1.216119
2	4	4	10	1.806479	1.161914	1.405856	1.257392	1.205315
2	4	6	10	1.75165	1.14377	1.459096	1.27073	1.195127
2	4	8	10	.	1.155278	1.417571	1.29196	1.252594
2	4	16	10	1.946763	1.186938	1.482485	1.339961	1.28408
2	4	22	10	2.210331	1.183049	1.523062	1.352596	1.241055
2	4	26	10	1.987438	1.156423	1.572596	1.36087	1.221038
2	4	30	10	1.591858	1.157287	1.529035	1.35227	1.222525
2	4	36	10	1.394739	1.150555	1.436376	1.301479	1.223893
2	4	0	12	1.302062	5.935832	1.355319	1.790649	1.559287
2	4	6	12	1.192902	4.853123	.	2.522414	1.919281
2	4	8	12	1.18619	4.769384	1.213724	2.531439	1.940834
2	4	16	12	1.186309	5.702854	.	2.781495	2.330966
2	4	22	12	1.20749	7.360661	1.13253	2.877327	2.374748
3	1	0	8	2.050802	1.110615	1.228244	1.197816	1.139158
3	1	2	8	2.000661	1.127666	.	1.229329	1.153824
3	1	4	8	1.822782	1.133578	.	1.270816	1.190124
3	1	6	8	1.791931	1.131789	.	1.290944	1.204568

3	1	8	8	1.791441	1.144448	.	1.309039	1.216254
3	1	12	8	1.778806	1.188292	1.462807	1.38384	1.272646
3	1	22	8	2.261733	1.173047	1.539647	1.410442	1.265037
3	1	26	8	2.128403	1.173682	1.685834	1.415995	1.34096
3	1	30	8	1.65843	1.155148	1.629226	1.387004	1.308021
3	1	36	8	1.116157	1.097074	1.164959	1.115625	1.130578
3	1	0	10	1.292155	4.680447	1.39039	1.767206	1.44266
3	1	2	10	1.076971	4.284485	.	1.970052	1.735008
3	1	4	10	1.195127	0.879479	.	2.15084	1.829644
3	1	6	10	1.18805	3.819278	.	2.215748	1.89828
3	1	12	10	1.178469	4.272799	1.139529	2.409268	1.96106
3	1	16	10	1.184583	4.830365	.	2.469121	2.061888
3	1	22	10	1.205409	6.214606	1.157797	2.665636	2.132317
3	1	26	10	1.176764	5.293152	1.192331	2.749355	2.267041
3	1	30	10	1.156165	3.351968	1.163921	2.660955	2.222678
3	1	36	10	1.148476	2.49924	1.123651	2.25296	2.090892
3	1	0	12	1.177412	1.923202	1.326157	1.711831	1.814749
3	1	2	12	1.15928	1.643381	1.327912	1.587968	1.599324
3	1	4	12	1.137046	1.465427	1.261133	1.494421	1.491859
3	1	6	12	1.129668	1.38834	1.278015	1.42508	1.449881
3	1	8	12	1.127638	1.373995	1.280972	1.376017	1.419654
3	1	12	12	1.131572	1.298845	1.304335	1.311562	1.361456
3	1	16	12	1.124577	1.271565	1.31334	1.273091	1.352758
3	1	22	12	1.137469	1.335942	1.392953	1.25305	1.363773
3	2	0	8	1.132657	5.611691	1.11259	1.525118	1.393274
3	2	2	8	1.130928	5.621884	.	1.727762	1.562005
3	2	4	8	1.118079	4.855734	.	2.085582	1.718479
3	2	6	8	1.122245	4.606288	.	2.281889	1.862517
3	2	8	8	1.130723	4.486742	.	2.360052	1.993057
3	2	12	8	1.15902	4.580998	1.047978	2.464686	2.009035
3	2	22	8	1.18706	7.272738	1.089331	2.576461	2.265215
3	2	26	8	1.151867	6.40107	1.172279	2.764492	2.318743
3	2	30	8	1.140662	4.043994	1.136495	2.869646	2.2073
3	2	36	8	1.136971	2.720611	1.128094	2.36106	2.029342
3	2	0	10	1.145516	2.216045	1.384037	1.849818	1.823314
3	2	2	10	1.129205	1.837254	1.345728	1.708865	1.647378
3	2	4	10	1.123758	1.599737	1.337997	1.602211	1.520726
3	2	8	10	1.123164	1.40798	1.311657	1.471378	1.420616
3	2	12	10	1.140598	1.361989	1.368331	1.422637	1.425437
3	2	16	10	1.122701	1.296382	1.336041	1.321407	1.334082
3	2	22	10	1.152901	1.250305	1.497237	1.252458	1.26917
3	2	26	10	1.127413	1.205294	1.371795	1.234331	1.241288
3	2	30	10	1.117983	1.154828	1.233404	1.183854	1.180997

3	2	36	10	1.117919	1.169854	1.174922	1.215763	1.261696
3	2	0	12	1.914942	1.178623	1.226453	1.198174	1.214461
3	2	2	12	1.801402	1.134441	1.371496	1.232866	1.186581
3	2	4	12	1.713802	1.1333	1.461035	1.274905	1.180039
3	2	6	12	1.663358	1.145271	1.495924	1.298481	1.185919
3	2	8	12	1.648418	1.15658	1.48375	1.313494	1.204463
3	2	12	12	1.681037	1.137188	1.430847	1.308739	1.207113
3	2	16	12	1.860779	1.160989	1.505412	1.332893	1.230727
3	2	22	12	2.136025	1.277289	1.475626	1.387203	1.340978
3	3	0	8	2.205561	1.185842	1.209258	1.165572	1.156331
3	3	2	8	2.103566	1.147773	.	1.235683	1.181937
3	3	4	8	1.885211	1.137978	.	1.266746	1.186409
3	3	6	8	1.813462	1.147623	.	1.280927	1.197495
3	3	8	8	1.805306	1.149728	.	1.291655	1.212091
3	3	12	8	1.805267	1.203492	1.406679	1.372181	1.278512
3	3	22	8	2.334536	1.272128	1.479086	1.419272	1.360464
3	3	26	8	2.181792	1.194396	1.648475	1.448996	1.357674
3	3	30	8	1.660098	1.16791	1.522063	1.388757	1.281937
3	3	36	8	1.391917	1.150332	1.419386	1.304667	1.247483
3	3	0	10	1.295064	1.187814	1.597063	1.273211	1.287739
3	3	2	10	1.227207	1.133879	1.500735	1.229792	1.202476
3	3	4	10	1.186126	1.126487	1.445969	1.199936	1.172107
3	3	8	10	1.161749	1.11781	1.408214	1.170967	1.145654
3	3	12	10	1.138548	1.127117	1.433065	1.17328	1.171562
3	3	16	10	1.149189	1.115815	1.447762	1.148742	1.137821
3	3	22	10	1.148907	1.139647	1.50745	1.161113	1.172636
3	3	26	10	1.134896	1.131627	1.351333	1.187309	1.196466
3	3	30	10	1.123253	1.105121	1.219258	1.126664	1.12418
3	3	36	10	1.118348	1.111626	1.17324	1.126482	1.143033
3	3	0	12	1.140928	6.45105	1.176099	1.497936	1.455635
3	3	2	12	1.123055	5.318014	1.17203	1.8691	1.638635
3	3	4	12	1.123444	5.002363	1.153649	2.223369	1.791128
3	3	6	12	1.138883	4.860949	1.058751	2.348284	1.929872
3	3	8	12	1.14893	4.853456	1.103679	2.440174	2.056461
3	3	12	12	1.168401	5.098193	1.060837	2.548163	2.12599
3	3	16	12	1.167533	6.021562	1.025034	2.557439	2.32604
3	3	22	12	1.190431	8.021688	1.117839	2.692566	2.539546
3	4	0	8	1.132014	1.101735	1.380997	1.100536	1.098265
3	4	2	8	1.138791	1.096369	.	1.111101	1.108103
3	4	4	8	1.114565	1.092934	.	1.101904	1.097643
3	4	6	8	1.110196	1.092651	.	1.100085	1.095718
3	4	8	8	1.109305	1.092861	.	1.099231	1.095294
3	4	12	8	1.132373	1.101697	1.368062	1.122312	1.122334

3	4	22	8	1.12334	1.096014	1.51529	1.109266	1.108347
3	4	26	8	1.116834	1.092421	1.373269	1.098865	1.099738
3	4	30	8	1.112513	1.092752	1.259563	1.095142	1.096636
3	4	36	8	1.119416	1.096121	1.18683	1.111405	1.111989
3	4	0	10	2.159649	1.125633	1.235443	1.166864	1.134789
3	4	2	10	1.955842	1.119942	1.464676	1.242559	1.156242
3	4	4	10	1.824664	1.12555	1.463328	1.294675	1.16989
3	4	6	10	1.784867	1.147551	1.434075	1.318062	1.186947
3	4	12	10	1.787523	1.137847	1.405457	1.327425	1.204289
3	4	16	10	2.093536	1.165264	1.476926	1.353939	1.240053
3	4	22	10	2.407135	1.16822	1.483317	1.368799	1.270725
3	4	26	10	2.203225	1.157985	1.668457	1.394638	1.269786
3	4	30	10	1.646609	1.157178	1.539219	1.379345	1.257558
3	4	36	10	1.386001	1.146643	1.391066	1.303734	1.229764
3	4	0	12	1.329815	5.743559	1.35745	1.580226	1.527091
3	4	2	12	1.245759	4.890581	1.307028	1.88045	1.601467
3	4	4	12	1.216501	4.587727	1.16578	2.090376	1.740392
3	4	6	12	1.186293	4.385235	1.18837	2.221219	1.856936
3	4	8	12	1.188437	4.353593	1.171148	2.286513	1.950486
3	4	12	12	1.193343	4.604934	1.120214	2.429108	2.086638
3	4	16	12	1.207857	5.151027	1.076457	2.301545	2.145039
3	4	22	12	1.210811	6.618733	1.153035	2.322439	2.239493
4	1	0	8	1.124012	5.06716	1.12374	1.324967	1.308945
4	1	2	8	1.118477	5.217832	1.090333	1.570897	1.49325
4	1	4	8	1.118904	4.771725	1.097726	1.761891	1.544811
4	1	6	8	1.126817	4.529323	1.037567	1.947896	1.647296
4	1	8	8	1.133247	4.515543	1.097799	2.074376	1.755482
4	1	12	8	1.156021	4.801609	1.073452	2.232538	1.887096
4	1	16	8	1.160612	5.738704	1.072869	2.19662	1.996252
4	1	22	8	1.18851	7.310196	1.071156	2.350155	2.176563
4	1	26	8	1.169815	7.135756	1.164693	2.516618	2.259659
4	1	30	8	1.149808	4.468937	1.144286	2.762573	2.256285
4	1	36	8	1.155924	3.084604	1.149342	2.408496	2.110003
4	1	0	10	2.026726	2.389285	1.187708	1.834217	1.856718
4	1	2	10	2.087376	2.033741	1.345087	1.764949	1.637019
4	1	12	10	1.796438	1.410486	1.413184	1.549015	1.450374
4	1	16	10	2.001846	1.399887	1.443398	1.500369	1.429046
4	1	22	10	2.272761	1.35379	1.464892	1.447152	1.422882
4	1	26	10	2.040055	1.246034	1.504093	1.440713	1.289272
4	1	30	10	1.657153	1.238377	1.523496	1.408937	1.34521
4	1	36	10	1.413095	1.201454	1.432404	1.322307	1.30188
4	1	2	12	1.229068	1.146336	1.530674	1.242499	1.183799
4	1	4	12	1.202871	1.150773	1.382911	1.224199	1.163563

4	1	6	12	1.186152	1.18136	1.383074	1.23672	1.177504
4	1	8	12	1.136046	4.205277	1.13371	2.281115	1.690982
4	1	12	12	1.166418	1.125606	1.355029	1.180348	1.150576
4	1	16	12	1.153302	1.133119	1.430201	1.16344	1.150203
4	1	22	12	1.15676	1.121386	1.46851	1.145537	1.133802
4	2	0	8	2.069653	1.179941	1.225704	1.209887	1.148007
4	2	2	8	1.880855	1.1328	1.409231	1.282916	1.143453
4	2	4	8	1.797125	1.128317	1.433794	1.323599	1.148639
4	2	6	8	1.769252	1.138969	1.442986	1.340717	1.161589
4	2	8	8	1.794842	1.14452	1.493711	1.351405	1.169987
4	2	12	8	1.846783	1.1917	1.479164	1.411644	1.260316
4	2	16	8	2.161008	1.154478	1.563182	1.419821	1.2034
4	2	22	8	2.363921	1.27511	1.545093	1.487244	1.31641
4	2	26	8	2.220065	1.225406	1.658304	1.5192	1.365733
4	2	30	8	1.594781	1.165712	1.600039	1.422641	1.257939
4	2	36	8	1.401851	1.152972	1.506222	1.330163	1.251671
4	2	0	10	1.274579	1.1608	1.523183	1.257396	1.236591
4	2	2	10	1.131126	1.641807	1.360025	1.513918	1.452838
4	2	12	10	1.167425	1.121425	1.376054	1.167098	1.160707
4	2	16	10	1.1235	1.262611	1.300548	1.2576	1.27716
4	2	22	10	1.163083	1.118715	1.456842	1.147204	1.151676
4	2	26	10	1.135426	1.111819	1.327349	1.131737	1.136652
4	2	30	10	1.123864	1.101489	1.228407	1.113012	1.113587
4	2	36	10	1.118154	1.102032	1.173497	1.10829	1.109286
4	2	0	12	1.146277	5.453408	1.165903	1.740903	1.381425
4	2	2	12	1.138017	4.669289	1.125254	1.912945	1.48234
4	2	4	12	1.132541	4.287153	1.06812	2.141632	1.570153
4	2	6	12	1.138202	4.183516	1.081455	2.272371	1.638781
4	2	8	12	1.173227	1.178049	1.337351	1.232981	1.180696
4	2	12	12	1.152611	4.40167	1.088746	2.458616	1.77252
4	2	16	12	1.161626	4.96774	1.101264	2.504528	1.857643
4	2	22	12	1.180796	6.484118	1.133446	2.693784	2.006142
4	3	0	8	1.121939	4.506932	1.12298	1.411036	1.317136
4	3	2	8	1.113556	4.187417	1.084566	1.63114	1.398571
4	3	4	8	1.12102	4.09253	1.060188	1.851072	1.469401
4	3	6	8	1.132216	4.137548	1.079736	1.984887	1.567513
4	3	8	8	1.130782	4.295768	1.09595	2.073415	1.659806
4	3	12	8	1.162538	4.57673	1.05936	2.236256	1.755653
4	3	16	8	1.159013	5.320048	1.042505	2.224627	1.865631
4	3	22	8	1.20907	6.814702	1.092353	2.300047	1.996677
4	3	26	8	1.165177	5.583566	1.156048	2.364178	2.067758
4	3	30	8	1.146771	3.774406	1.132019	2.499847	2.005809
4	3	36	8	1.153558	2.547236	1.129983	2.089011	1.823946

4	3	0	10	1.156481	1.996829	1.341934	1.634166	1.676311
4	3	2	10	1.203787	1.149556	1.47318	1.218552	1.163329
4	3	12	10	1.131226	1.27703	1.309508	1.285771	1.283672
4	3	16	10	1.141056	1.117169	1.376934	1.146935	1.136209
4	3	22	10	1.134418	1.207925	1.454413	1.207368	1.229439
4	3	26	10	1.130904	1.179769	1.327563	1.213195	1.213577
4	3	30	10	1.124468	1.146129	1.220537	1.161578	1.156255
4	3	36	10	1.109806	1.128175	1.163081	1.135063	1.142132
4	3	0	12	2.141911	1.144841	1.226763	1.176084	1.152478
4	3	2	12	1.912628	1.128262	1.388976	1.234925	1.159436
4	3	4	12	1.786835	1.116535	1.398827	1.29028	1.167809
4	3	6	12	1.759387	1.138202	1.424697	1.306323	1.170502
4	3	8	12	1.745414	1.145014	1.45185	1.323533	1.181251
4	3	12	12	1.465613	1.255855	1.344779	1.387164	1.297278
4	3	16	12	1.932504	1.146095	1.452915	1.326966	1.199176
4	3	22	12	2.323089	1.234221	1.445933	1.390551	1.294524
4	4	0	8	1.959291	1.188702	1.174918	1.150078	1.132262
4	4	2	8	1.75328	1.176801	1.365546	1.223814	1.137465
4	4	4	8	1.717445	1.138523	1.395913	1.264236	1.137684
4	4	6	8	1.693149	1.216044	1.404194	1.304568	1.155413
4	4	8	8	1.710373	1.146073	1.407932	1.295425	1.15734
4	4	12	8	1.761801	1.171214	1.386939	1.348366	1.228105
4	4	16	8	1.985675	1.154696	1.455988	1.303208	1.21246
4	4	22	8	2.399431	1.172543	1.499184	1.323379	1.214234
4	4	26	8	2.150026	1.156464	1.554692	1.348387	1.203972
4	4	30	8	1.558944	1.160189	1.489381	1.339297	1.221109
4	4	36	8	1.34777	1.135987	1.372325	1.281716	1.190413
4	4	0	10	1.275143	6.716109	1.2981	1.56208	1.515759
4	4	2	10	1.22377	5.915516	1.246181	1.884445	1.68973
4	4	12	10	1.1764	5.64751	1.127134	2.541442	2.154855
4	4	16	10	1.192408	6.540988	1.159868	2.45901	2.216584
4	4	22	10	1.193713	8.660266	1.170505	2.444844	2.401943
4	4	26	10	1.172123	6.497598	1.132763	2.631378	2.289038
4	4	30	10	1.159752	4.41199	1.11272	2.804289	2.292651
4	4	36	10	1.153573	3.04544	1.119111	2.331063	2.224238
4	4	0	12	1.310323	6.516158	1.313474	1.401764	1.50413
4	4	2	12	1.136613	1.713517	1.317324	1.591963	1.518392
4	4	4	12	1.114451	1.553681	1.283596	1.502425	1.390744
4	4	6	12	1.128114	1.457974	1.276266	1.450122	1.372969
4	4	8	12	1.131612	1.376541	1.262152	1.390502	1.352738
4	4	12	12	1.42207	1.209889	1.346289	1.327074	1.25937
4	4	16	12	1.124906	1.277027	1.311426	1.258447	1.277902
4	4	22	12	1.159162	1.252199	1.39927	1.240634	1.261257

1	1	24h	8	1.102163	1.092149	1.333661	1.094285	1.092634
1	1	24h	10	1.146704	5.735327	1.137317	2.443493	2.127704
1	1	24h	12	1.951167	1.541857	1.492555	1.657378	1.576814
1	2	24h	8	2.136243	1.134222	1.387748	1.304609	1.221011
1	2	24h	10	1.176381	1.107796	1.435947	1.163728	1.137487
1	2	24h	12	1.162441	5.657516	1.16755	2.464062	2.1131
1	3	24h	8	1.107132	1.094521	1.434043	1.097579	1.094902
1	3	24h	10	1.387353	4.306439	1.234197	2.264411	1.765673
1	3	24h	12	2.01172	1.468332	1.433222	1.584542	1.518861
1	4	24h	8	1.110715	1.09077	1.358339	1.100241	1.094646
1	4	24h	10	1.849955	2.35113	1.369758	1.789469	1.479057
1	4	24h	12	1.21858	6.067998	1.221291	2.03805	2.165284
2	1	24h	8	1.107663	1.091275	1.357949	1.097475	1.095167
2	1	24h	10	1.156421	5.384729	1.155338	2.1321	2.000601
2	1	24h	12	2.046778	1.449406	1.488184	1.543105	1.522044
2	2	24h	8	2.038858	1.168625	1.404871	1.281617	1.243614
2	2	24h	10	1.217413	6.028532	1.21288	2.514797	2.17258
2	2	24h	12	1.133361	1.411449	1.321348	1.401512	1.395085
2	3	24h	8	1.925853	1.143419	1.371801	1.247547	1.213236
2	3	24h	10	1.17212	1.11208	1.373842	1.158439	1.135461
2	3	24h	12	1.15596	6.292292	1.160683	2.480373	2.185402
2	4	24h	8	1.106926	1.095672	1.358989	1.095118	1.095346
2	4	24h	10	1.966293	1.203163	1.454918	1.320454	1.288831
2	4	24h	12	1.197815	5.77497	1.229199	2.698884	2.082349
3	1	24h	8	2.064401	1.180539	1.506079	1.378304	1.293305
3	1	24h	10	1.207872	4.768627	1.253135	2.412793	1.978114
3	1	24h	12	1.12581	1.359654	1.303805	1.366604	1.422279
3	2	24h	8	1.144209	5.26227	1.15487	2.421642	2.067533
3	2	24h	10	1.12324	1.399641	1.429165	1.423021	1.404559
3	2	24h	12	1.918892	1.161414	1.49427	1.298428	1.25652
3	3	24h	8	2.084191	1.217736	1.457531	1.3559	1.334183
3	3	24h	10	1.16567	1.11057	1.437244	1.158595	1.138096
3	3	24h	12	1.16268	5.966745	1.168032	2.51302	2.189699
3	4	24h	8	1.107306	1.090359	1.408026	1.099425	1.095071
3	4	24h	10	2.110668	1.151934	1.462783	1.321609	1.241581
3	4	24h	12	1.208803	5.205853	1.24529	2.411743	2.153847
4	1	24h	8	1.1488	5.390283	1.149102	2.260273	1.97887
4	1	24h	10	2.096416	1.54338	1.466455	1.587825	1.53853
4	1	24h	12	1.168199	1.120947	1.401611	1.176766	1.148463
4	2	24h	8	2.097021	1.22658	1.530972	1.395289	1.307849
4	2	24h	10	1.151243	1.108573	1.43226	1.157903	1.132536
4	2	24h	12	1.161045	5.042335	1.165142	2.490291	1.841533
4	3	24h	8	1.153884	4.916569	1.145656	2.172101	1.773051

4	3	24h	10	1.122679	1.342209	1.308488	1.311326	1.307057
4	3	24h	12	1.987303	1.217568	1.460134	1.357756	1.298728
4	4	24h	8	2.004805	1.193814	1.43155	1.306752	1.252126
4	4	24h	10	1.192322	6.408719	1.218562	2.483716	2.171404
4	4	24h	12	1.128571	1.393597	1.291544	1.357459	1.35656

Treatment Assignment

	Treatments*			
Fermenters	Period 1	Period 2	Period 3	Period 4
1	InfAc	InfPr	LowpH	Control
2	InfPr	Control	InfAc	LowpH
3	LowpH	InfAc	Control	InfPr
4	Control	LowpH	InfPr	InfAc

*InfAc: Acetate infusion @ 20 mmol/d; InfPr: Propionate infusion @ 7mmol/d; LowpH: pH lowered by adjusting buffer composition.

Isotope Sequence

	Period 1				Period 2			Period 3			Period 4		
Treatments	d 8	d 10	d 12	d 14	d 8	d 10	d 12	d 8	d 10	d 12	d 8	d 10	d 12
InfAc	Bu	Pr	Ac	UBu	Ac	Bu	Pr	Pr	Bu	Ac	Ac	Pr	Bu
InfPr	Ac	Bu	Pr	UBu	Bu	Pr	Ac	Bu	Ac	Pr	Pr	Bu	Ac
LowpH	Bu	Pr	Ac	UBu	Bu	Ac	Pr	Ac	Pr	Bu	Ac	Bu	Pr
Control	Bu	Ac	Pr	UBu	Ac	Pr	Bu	Ac	Bu	Pr	Pr	Ac	Bu

*Ac= 2-^{13}C sodium acetate; Pr=13C$_3$ sodium propionate; Bu=1-^{13}C sodium butyrate; UBu= 13C$_4$ sodium butyrate