NUTRIENT ANALYSIS OF WATER BUFFALO MILK AND WATER BUFFALO MILK ACID WHEY: A PILOT STUDY TO EVALUATE SUITABILITY AS A PROTEIN SUPPLEMENT FOR HUMAN CONSUMPTION IN LAO PDR

By

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CERTIFICATE OF APPROVAL

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Abstract

Background: In Laos, 33.5% of children under five years of age are stunted, 26% are underweight and 8% are severely wasted. Additionally, the proportion of underweight adults living in Laos is 9.8%. Nutritional rehabilitation of malnourished children and adults often requires dietary energy, protein, and micronutrient supplementation. Water buffalo, native to Southeast Asia, produce milk with higher energy, protein and micronutrient concentrations than dairy cow's milk. Acid whey, a byproduct of soft-cheese production, is currently discarded at the Laos Buffalo Dairy in Laos after making cheese. The total energy and macronutrient concentrations of Laos water buffalo milk and acid whey were analyzed and compared to dairy cow's milk and acid whey data from Egypt and the US, respectively, to determine their suitability as a dietary energy, protein, and micronutrient supplement.

Methods: Raw, unpasteurized water buffalo milk and liquid whey samples were obtained from the Laos Buffalo Dairy before and after making feta, mozzarella and ricotta cheese. Nutrient analyses were performed by the Institute of Nutrition at Mahidol University, Bangkok, Thailand. Crude protein concentrations were analyzed using the Kjeldahl method, fat concentrations were measured using Soxtec™ technology, and carbohydrate concentrations were determined as a difference in weight of 100 grams of milk and the measured weight of each milk constituent. Energy contents were then determined by back calculation. Lactose concentrations were measured with a refractive index, moisture and ash concentrations were determined using gravimetric techniques, and calcium was determined using flame atomic absorption spectrometry.

Because all liquid mozzarella whey is used to make ricotta cheese, feta and ricotta whey nutrient concentrations were mathematically combined to reflect a pooled acid whey available to create a dietary supplement.

Results: Mean energy (107 kcal/100g), protein (4.5g/100g), and fat (7.6g/100g) concentrations in water buffalo milk were 51%, 29%, and 85% higher than reported in Egyptian dairy cow's milk, respectively. The concentrations of total energy in water buffalo liquid feta, mozzarella, ricotta, and a mixture of feta and ricotta whey were 31%, 27%, 13%, and 22% higher than reported in US dairy cow's milk whey, respectively. Water buffalo liquid and dried acid whey contained higher concentrations of crude fat than dairy cow's liquid and dried acid whey. The concentration of protein in water buffalo dried feta and mozzarella whey was similar to dried dairy cow's whey, while the concentration of protein in dried ricotta whey was 55% lower than dried dairy cow's whey. Water buffalo dried feta, mozzarella, and an equal-parts mixture of dried feta and ricotta acid whey contained at least 7 grams of protein per 100 grams of powdered whey and were classified as suitable dietary protein sources.

Conclusions: Laos water buffalo milk contains higher concentrations of energy, protein and fat than dairy cow's milk. Dried water buffalo feta, mozzarella, and an equal-parts mixture of feta and ricotta whey meet the protein standards of an acceptable dried whey. These results suggest that Laos water buffalo milk and its acid whey fraction would be a suitable source of dietary energy, protein, and other nutrients.

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CHAPTER 1

Specific Aims

Lao People's Democratic Republic (Lao PDR) is a small, landlocked country in Southeast Asia, bordered by Myanmar, China, Vietnam, Thailand, and Cambodia. The country is classified as "developing", as defined by its status as a lower-middle income economy, and has identified nutrition as a top priority to achieve the country's Sustainable Development Goals.[1,2] Of children under five years of age living in Lao PDR, 33.5% are classified as stunted, 26% as underweight, and 8% as wasted.[3,4] In addition, the proportion of underweight adults living in Lao PDR is 9.8%.[5] Conditions that contribute to malnutrition and poor health outcomes in both children and adults include the lack of poor sanitation practices, low economic status, and living in remote mountain villages with limited access to adequate amounts and varieties of food, medical care, and sources of clean water.[1,6,7]

Traditional dietary practices in Lao PDR do not support the nutritional rehabilitation of malnourished children and adults who require high amounts of energy, protein, and other nutrients to recover from illness. The standard diet in Lao PDR is high in carbohydrate due to the ubiquitous consumption of polished sticky rice and, combined, glutinous sticky rice and steamed rice account for 77-83% of total daily energy intakes in the country.[8] In contrast, dietary fat and protein contents of traditional diets are often low. Typical sources of protein in Laotian diets include small portions of meat or fish,[8] whereas fat is provided by animal lard and vegetable oils.[9] One potential, locally-derived, sustainable source of energy, protein, and other nutrients, that tends not to be consumed in Lao PDR, is water buffalo milk or the whey fraction of water buffalo milk produced during the cheese manufacturing process.

Water buffalo are native to Southeast Asia and are an ideal livestock in Lao PDR as they are resistant to parasites; thrive in hot, tropical climates; and have lower nutrient requirements than dairy cattle.[10,11] Water buffalo have historically been used to till and plant rice fields; however, with the recent availability of motor-powered tillers, water buffalo are now raised to provide families with a living “savings account” that can be sold in times of economic distress. In other Asian countries, such as India and Pakistan, water buffalo are used as the primary source of milk for human consumption.[12,13] Compared to cow’s milk, water buffalo milk is higher in protein and fat, and as a result, is more calorically dense.[10] Although water buffalo are common throughout Lao PDR, milking water buffalo and consuming their milk is a novel concept to many farmers and families.

While dietary sources of protein are included in the traditional diet in Lao PDR, these foods are perishable and are not always available to families living in remote areas. Therefore, a shelf-stable, locally-sourced protein/micronutrient supplement that will support the physical growth and cognitive development of children and rehabilitation of malnourished adults is needed. During childhood, protein requirements, expressed in grams per kilogram, are higher than in adulthood to ensure sufficient amino acid substrates to synthesize hormones, transport proteins, etc., and for tissue expansion needed for growth.[14,15] Among patients who are critically ill, protein needs are 2-3 times higher than the recommended amount for healthy individuals to reduce muscle loss and maximize healing.[16] Powdered cow's milk whey protein is often used in the United States as a source of protein, or protein modulator, in the diet of critically ill patients. Whey protein is of high biological value and contains high amounts of essential amino acids that are important for protein synthesis.[17,18]

Currently, the Laos Buffalo Dairy, located in Luang Prabang, Lao PDR and established in 2016, is raising water buffalo for milk production to make soft cheeses and other dairy products for retail and commercial sales. At this time, the acid whey fraction of water buffalo milk, a byproduct of the cheese-making process, is discarded. However, this waste product may be a suitable source of energy and protein to support the nutritional rehabilitation of malnourished children and adults. At this time, there is no published data describing the nutritional content of Lao PDR water buffalo milk or the acid whey fraction of water buffalo milk generated in the soft cheese-making process. To address this gap, nutritional analyses of water buffalo milk and its liquid and dried acid whey fraction are needed. These nutritional analyses will determine if Lao PDR water buffalo milk and its acid whey derived during the cheese manufacturing process contain sufficient amounts of energy and protein to serve as a nutritional supplemental for malnourished children and adults.

The goals of this research were to determine the nutritional composition of water buffalo milk and liquid and reconstituted dried acid whey samples generated from the cheese making process at the Laos Buffalo Dairy in Luang Prabang, Lao PDR. Based on the results, longer-term goals of this research are to: 1) identify a process to filter, powder, and package the whey protein; 2) scale-up the production of a shelf-stable, powdered whey protein supplement; and 3) determine if incorporation of this powdered protein supplement into the diets of malnourished children and adults improves their nutritional status and health outcomes.

Specifically, this research aimed to:

Aim 1: Determine the energy, macronutrient, moisture, ash, and calcium concentrations of raw, unpasteurized Lao PDR water buffalo milk and compare these values to available reference data derived from raw, unpasteurized cow's milk in Egypt.[19]

Aim 2: Determine the energy, macronutrient, moisture, and ash composition of the liquid acid whey fraction of pasteurized Lao PDR water buffalo milk produced as a part of the soft cheese-making process and compare these values to reference data of liquid acid whey derived from cow's milk as reported in the United States Department of Agriculture (USDA) FoodData Central nutrient database.[20]

Aim 3: Determine the energy, macronutrient, moisture, and ash composition of the reconstituted dried acid whey fraction of pasteurized Lao PDR water buffalo milk produced as a part of the soft cheese-making process and compare these values to reference data of dried acid whey derived from cow's milk in the USDA FoodData Central nutrient database.[21]

To accomplish these aims, the following hypotheses were tested:

<u>Hypothesis 1:</u> Raw, unpasteurized Lao PDR water buffalo milk will have higher concentrations of energy, protein, fat, carbohydrate, lactose, calcium, and ash and a

lower moisture content than what has been reported for raw, unpasteurized dairy cow's milk in Egypt.[19]

Hypothesis 2 (a): Pasteurized Lao PDR water buffalo milk liquid acid whey will have higher concentrations of energy, fat, carbohydrate, and ash and a lower moisture content than what has been reported for dairy cow's milk liquid acid whey as reported in the USDA FoodData Central nutrient database.[21]

Hypothesis 2 (b): The protein concentration of pasteurized Lao PDR water buffalo milk liquid acid whey will be at least 0.76 grams of protein per 100 grams of liquid acid whey and will meet the protein concentration standard of liquid acid whey derived from dairy cow's milk as reported by the United States Dairy Export Council.[22]

Hypothesis 3 (a): Pasteurized Lao PDR water buffalo milk reconstituted dried acid whey will have higher concentrations of energy, fat, carbohydrate, and ash than dairy cow's milk reconstituted dried acid whey as reported in the USDA FoodData Central nutrient database.[21]

Hypothesis 3 (b): The protein concentration of pasteurized Lao PDR water buffalo milk reconstituted dried acid whey will be at least 7 grams of protein per 100 grams of powdered acid whey or 7% by weight volume, thereby aligning with the protein concentration of powdered acid whey derived from dairy cow's milk as reported by the United States Dairy Export Council.[22]

Determining the nutritional composition of Lao PDR water buffalo milk and its liquid and reconstituted powdered acid whey byproducts, generated as part of the cheese making process, are the first steps to enable the development of a locally sourced, shelf-stable, powdered energy and protein supplement that is safe for human consumption and to use to rehabilitate malnourished children and adults.

CHAPTER 2

Background

Lao People's Democratic Republic (Lao PDR) is a small, landlocked country in Southeast Asia, bordered by Thailand, Myanmar, China, Vietnam, and Cambodia (refer to Figure 1). The country is classified as "developing", as defined by its status as a lower-middle income economy, and has identified nutrition as a top priority to achieve the country's Sustainable Development Goals.[1,2]

Figure 1: Map of Lao PDR

In addition, the Global Hunger Index categorizes hunger and undernutrition in Lao PDR as “serious”, meaning that the lack of food, diet quality, child caregiving practices, and the overall health of the country is concerning.[2] In Lao PDR, 20.9% of the population lives in “serious” conditions that contribute to hunger and undernutrition,[3] and in 2018, of children under five years of age in Lao PDR, 33.5% were classified as stunted, 26% as underweight, and 8% as wasted.[3,4] Further, in 2018, 47% of the adult patients admitted to one of two national hospitals located in Vientiane, Lao PDR, were classified as being malnourished,[23] and the proportion of underweight adults living in Lao PDR is 9.8%.[5] Conditions that contribute to malnutrition and poor health outcomes in both children and adults in Lao PDR stem from poverty, insufficient access to clean water, poor sanitation practices, food insecurity, lack of dietary diversity, and geographical isolation from medical care.[1]

Malnutrition in Lao PDR

Poverty in Lao PDR

Low wages are one barrier preventing families from accessing nutritionally dense foods. In 2017, the average salary including in-kind transfers; meaning any food, clothing, housing, water, fuel, electricity, or transportation given freely or at a subsidized rate; and secondary job earnings was ₭2.5 million Lao Kip per month, or approximately $170 United States dollars per month.[24] Of the total population, 50% earned between ₭1-2 million Lao Kip per month ($112-$225 United States dollars per month) and 12% made less than ₭1 million Lao Kip per month.[24] Location affects the average household income as well. While 33% of urban dwellers earn less than ₭1.5

million Lao Kip per month, 43% of rural dwellers earn less than this amount.[24] Lao PDR's national poverty line is approximately ₭11,050 Lao Kip or $1.25 United States dollars per person per day.[1] With an average household size of six members, 30% of the population lives below the poverty line.[1,25] Overall, depending on location and monthly wages, 17% to 95% of households in Lao PDR cannot afford nutritious foods[26] (see Figure 2).[27] Low wages not only affect dietary adequacy and diversity, but prevent families from traveling to seek medical care. If medical services are available, low wages can often hinder families from affording this care.[28]

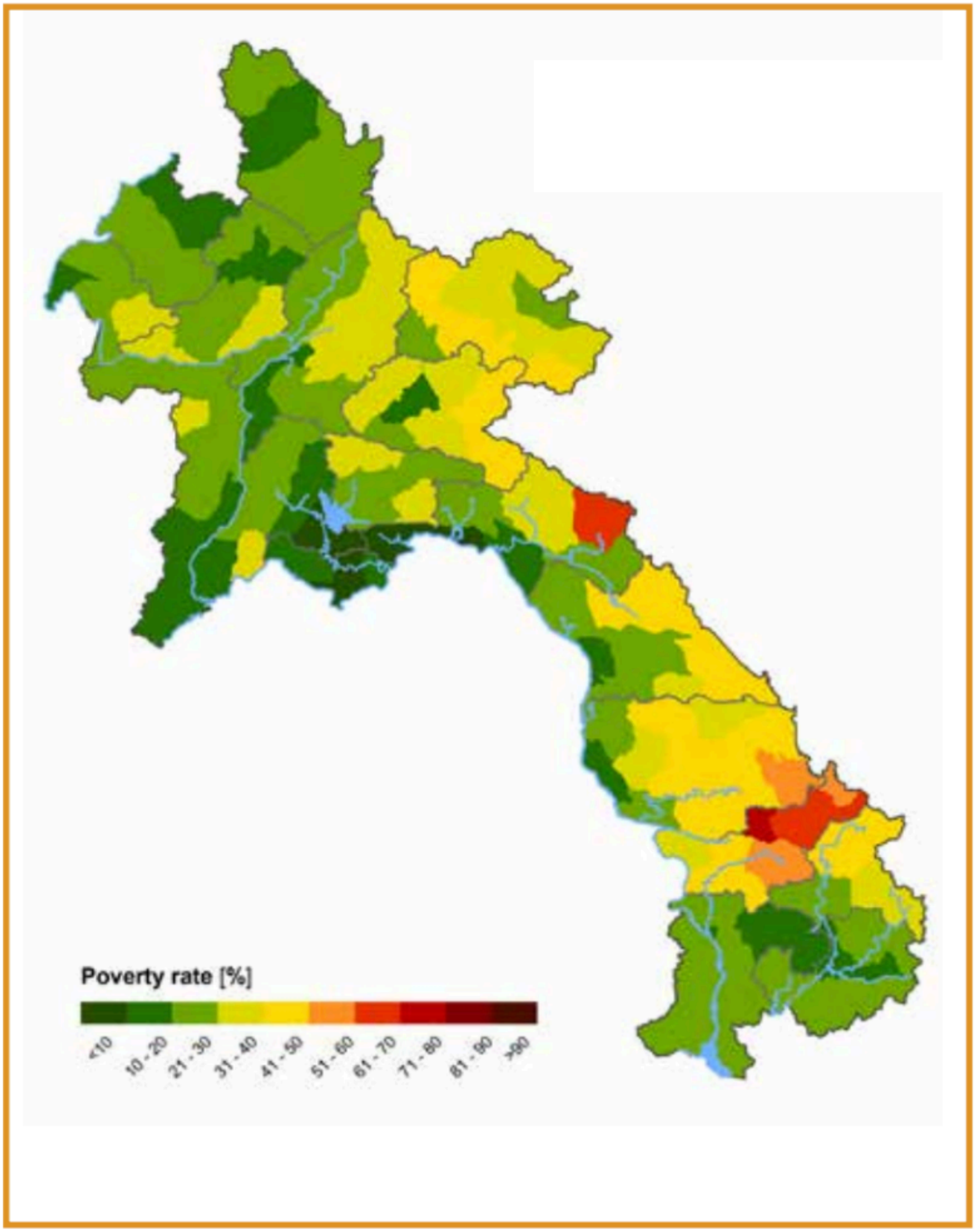

Figure 2: 2015 Lao PDR District Level Poverty Map[25]

*Poverty in Lao PDR is defined as earning less than ₭11,050 Lao Kip ($1.25 United States dollars) per person per day and does not meet the basic needs to live.

Water Quality and Sanitation in Lao PDR

Lack of clean water and poor sanitation practices also contribute to malnutrition.[7,28] Urban locations in Lao PDR have greater access to clean water within homes than rural locations. To compare, 26.7% of urban households have access to "on site" drinking water that is free of *Escherichia coli*, whereas 9.4% of rural locations have this same access.[4] Drinking water can be bought in rural and urban areas, but the cost hinders families from purchasing bottled water. Families can boil water for one minute to kill bacteria, viruses, or protozoa;[29] yet, knowledge about this practice is limited, especially in rural areas. Sanitation practices are also problematic. Approximately 80% of urban and 52% of rural populations have soap available when washing their hands.[4] Of the total population, 24% still practice open defecation.[7] Poor access to clean water within the home and poor sanitation practices contribute to food-borne illness outbreaks, diarrhea, and intestinal infections, while open defecation decreases access to clean water. Each of these conditions and situations contribute significantly to higher rates of malnutrition in children and adults.

Nutrient Deficiencies, Food Insecurity, and Dietary Practices in Lao PDR

Access to nutrient-rich foods in Lao PDR is often limited, and as a result, deficiencies in vitamin A, calcium, iron, B vitamins, and zinc can manifest.[6] These micronutrient deficiencies contribute to childhood stunting, infections, diarrhea, and micronutrient-related conditions such as beriberi (vitamin B-1 deficiency), anemia (iron and vitamin B-12 deficiency), and xerophthalmia (vitamin A deficiency). These conditions occur more commonly among vulnerable populations including infants, children, pregnant and lactating women, and the elderly.[6,30]

Food insecurity, or the lack of resources to acquire safe, consumable food, complicates the access to nutrient-rich foods. Rural and remote “upland” households in Lao PDR face the highest rates of food insecurity of 14% and 25%, respectively.[1] Food insecurity depends on the amount of food available, access to food, ability to purchase food, food utilization, and a person’s stability or vulnerability to food access.[31] Nearly 72% of the total population of Lao PDR receives their food from non-market sources, including family farms, foraging, bartering, or as gifts. In rural areas, non-market food sourcing rises to 83%.[8] Improving the livelihoods of farmers by increasing agricultural yields, diversifying crops grown, teaching farm coping mechanisms after severe weather conditions or unexpected natural disasters, and educating families about new farming techniques are priorities to improve the economic and physical capability of accessing food in Lao PDR. Increasing access to markets through improved infrastructures, roads, and market expansion are needed to increase the distribution and availability of food.[32]

Breastfeeding and weaning practices contribute to infant and child malnutrition and stunting.[28,33] Almost all children in Lao PDR are breasted to some extent, however, only 40% are exclusively breastfed for up to six months as recommended by the World Health Organization.[28,34] Inadequate energy, protein, fat, and micronutrient intakes of infants often begin within one week after birth as chewed sticky rice, water, diluted infant formulas, or sweetened condensed milk may be given in combination with breastfeeding.[28,33] In addition, maternal pre- and postpartum food avoidance practices, including limited intake or total avoidance of vegetables, fruits, meats, fermented foods, sauces, and spices, may reduce the production and nutritional content of breastmilk.[33] The inadequate volume of breastmilk provided in conjunction with depleted nutrient concentrations in breast milk place infants at risk for malnutrition.

Only 43% of children between the ages of 6-23 months receive enough solid foods, semi-solid foods, soft foods, or breast milk each day, or receive food frequently enough throughout the day.[28] Consuming infrequent meals is known as “minimum meal frequency”.[28,35] Infants and children between the ages of 6-23 months instead may be fed sticky rice with small amounts of fish and broth as complementary foods. These feeding practices contribute to “minimum dietary diversity”, defined as consuming food from fewer than five food groups.[28] Inadequate and inappropriate complementary feeding practices often stem from the need for women to return to work and a lack of knowledge of appropriate child feeding practices.[28]

Energy intake of a traditional Lao PDR diet is comprised of 77% sticky and steamed rice, which increases to 83% in rural locations.[8] In Lao PDR, as in much of Southeast Asia, rice is eaten at each meal in addition to small portions of higher protein foods and vegetables.[8] Most rice consumed in Lao PDR is glutinous, polished sticky rice. Glutinous, polished sticky rice is high in amylose and processed and prepared in a way that likely removes and/or destroys beneficial water-soluble vitamins, such as thiamin, or vitamin B1.[33] Cultural food practices in conjunction with very low access to nutrient-rich foods leaves many children unable to meet their physical and cognitive growth potential.

Access to Medical Care and Nutritional Interventions to Treat Malnutrition in Lao PDR

The Lao PDR healthcare system, which is comprised of three administration levels (central, provincial, and district), is a government-owned, country-wide program that encompasses both health care employees and community-health volunteers. Employed health care personnel work in health offices at each administration level, while community-health volunteers live in rural villages and offer first-line medical care.[36] Because rural subsistence farming is practiced by 66% of the total population[26], only basic drug kits, provided by the Lao PDR healthcare system to the village healthcare volunteers, are available to the majority of the country's population.[36] Access to higher-level medical care in Lao PDR is limited and often requires traveling long distances on unpaved roads in poor condition to reach district health centers, provincial hospitals, or national hospitals.

In addition to traveling long distances, payment for health services can be difficult. To assist in the payment for medical care, Lao PDR has a health insurance program supported by the tax-based National Health Insurance Scheme. The Lao PDR medical insurance system allows families to receive treatment at public health locations for a reduced out-of-pocket fee. Children under the age of five years, monks, poor families acknowledged by their village chief, and pregnant women are not required to pay for health care. Co-payments for health care often range from ₭5,000 to ₭20,000 Lao Kip, or $0.55 to $2.20 United States dollars per incident.[36,37] However, this amount is still difficult to pay for many Lao PDR citizens. The lack of both physical access and financial resources to pay for medical care to prevent or treat disease contributes to high rates of malnutrition in Lao PDR.

Another factor that may exacerbate malnutrition upon hospital admission is the lack of hospital foodservice within district, provincial, and national hospitals in Lao PDR. Instead, family members become caregivers responsible for providing food and hydration to their relative during hospitalizations, are often unable to afford nutritious foods or have access to a food preparation facility in or near the hospital. As a result, patients may consume inadequate diets when hospitalized. If specialized medical nutrition therapy is required during or after discharge from the hospital, such as enteral nutrition, families must make or pay for these formulas as well. Inadequate knowledge of healthy food preparation and sanitation practices, the lack of refrigeration, and decreased access to clean water make this practice of home preparation and administration of enteral formulas unsafe. Contamination of enteral formulas with harmful bacteria puts patients needing enteral nutrition therapy at risk for gastrointestinal infections that can result in diarrhea, dehydration, and sepsis, all of which can exacerbate pre-existing malnutrition.[38]

To support the cognitive and physical growth of malnourished children and rehabilitation of malnourished adults, a safe, shelf-stable and affordable source of energy, protein, and other nutrients that can easily be incorporated into a diet is needed. Cow's milk and whey protein derived from cow's milk is often used in the United States as an additional source of energy, protein, and other nutrients in the diet of malnourished children and adults. Therefore, a similar product, such as water buffalo milk and the whey protein derived from water buffalo milk, may be a realistic option for use in Lao PDR.

Mammalian Milk Composition

Milk is one of the defining characteristics of mammals and is produced by mammary secretory cells after childbirth.[39] In particular, animal milks are known to be good sources of dietary energy, protein, and fat for humans and contain a variety of vitamins and minerals including thiamin, riboflavin, vitamin B-12, vitamin A, calcium, magnesium, and pantothenic acid.[39-41] In general, mammalian milk is composed of 88.6% water; 4.5% to 5.2% lactose, a disaccharide; and 3.4% protein.[39] The fat component of milk, however, greatly varies between animal species and ranges between 3.5% to nearly 8%.[19,39] The nutrient concentrations in milk can vary and depend on the mammal species or breed, the number of previous lactation periods, and environmental factors such as diet.[39]

Lactose in Mammalian Milk

Lactose is the main carbohydrate in milk and is formed through a β-glycosidic bond between the two monosaccharides glucose and galactose. Lactose differs from other nutrients in milk in that it does not vary in concentration through changes in the mammal's environment. Instead, lactose concentration remains fairly constant.[39] The constant concentration of lactose is crucial when making yogurt or cheese, as lactose is needed as a substrate during the fermentation process of milk by lactic acid.[39]

When consumed, the first step in the digestive pathway of lactose is to break the disaccharide into two constituent monosaccharides; one molecule of glucose and one molecule of galactose. This process requires the gastrointestinal enzyme, lactase. It is estimated that 65% of the world's population has a deficiency in lactase and thus, has a reduced ability to digest lactose, or is lactose intolerant.[42] Milk consumption among those who are lactose intolerant often leads to gastrointestinal discomfort, and as a result, a decrease in milk consumption. However, lactose can be filtered from milk or lactase enzymes can be added to milk or taken orally to reduce the symptoms of lactose intolerance. Although it is not established what percentage of Lao PDR citizens have lactose intolerance, it is estimated that up to 90% of the people living in Southern Asia cannot digest lactose efficienty.[43] Regardless, when consumed in small amounts of up to 12 grams, the amount present in 8 ounces of milk, lactose can be tolerated by most individuals.[44]

Fat and Fat-Soluble Vitamins in Mammalian Milk

Fat is the main source of energy and fat-soluble vitamins in milk and presents as micelles suspended in the water component of milk.[39] As previously mentioned, the fat composition of milk varies drastically depending on mammalian species, breed, and environmental factors such as diet. Cow's milk fatty acids, in particular, are composed of approximately 70% saturated, 25% mono-unsaturated, 2.3% polyunsaturated, and 2.7% trans fatty acids.[39]

The fat present in animal milks can be categorized as "simple" or "complex". A "simple" lipid is a fatty acid that is either free or bound to glycerol through an ester linkage.[45] Triglycerides, which make up 96%-98% of the total lipids found in cow's milk, are comprised of three fatty acid chains bound to one glycerol molecule, making them a "simple" lipid.[39] In comparison, the second major class of lipids found in animal milks are phospholipids. Phospholipids make up 0.2% to 1% of cow's milk lipids and are classified as "complex" because they contain additional groups such as nitrogen or phosphorus bases on their fatty acid chains.[39,45] Other lipids constituents found in mammalian milk include cholesterol, free fatty acids, and cerebrosides.[39]

Fat is an important component of animal milk not only for the energy it provides, but also for its source of fat-soluble vitamins including vitamin A, E, and when fortified, vitamin D.[39] Vitamin A is crucial for maintaining eye health[30],while vitamin E is a component of cell membranes and acts as an antioxidant to protect cells from free-radical damage.[46] Vitamin D has a unique role in supporting and maintaining bone health by upregulating dietary calcium absorption in the small intestine and reabsorption in the kidney.[47] Unlike water-soluble vitamins, such as thiamin and riboflavin that are present in the water component of milk, fat soluble vitamins in milk are absorbed in the greatest concentrations when unskimmed, whole milk is consumed.[39]

Protein in Mammalian Milk

Proteins in milk are of high biological quality, meaning that they are comprised of sufficient amounts of all essential amino acids, and have a similar composition to the proteins in eggs.[39] The presence of all nine essential amino acids required for human life in mammal milks make milk a preferred "high biological value" source of protein.[39,48] This is important, as amino acids and the availability of nitrogen are essential during periods of growth to maintain and support developing muscle tissue.[22,49]

The total protein, or the protein content of milk that excludes non-protein nitrogen substances, determines the market value of milk.[39] For instance, milk containing a higher total protein content will yield more cheese, and therefore, would have a higher selling point. Although milk contains many different types of proteins, the two major proteins found in mammal milks are casein and whey. Casein accounts for 80% of the protein found in milk, whereas whey makes up the remaining 20%.[17,19,22] Often, whey protein that is used as a supplemental source of dietary protein is derived from cow's milk.

Whey protein derived from cow's milk is classified as a high biological value protein and contains similar vitamins and minerals to milk such as B-vitamins, calcium, vitamin A, magnesium, and pantothenic acid.[17,20,22,50] An alternative tool used to evaluate the quality of protein is the Protein Efficiency Ratio (PER). The PER is an index of weight gained by an individual divided by total amount of protein consumed. Whey protein has a PER of 3.2 g/g, meaning that for every gram of weight gained, 3.2 grams of whey was required to support that growth. A PER above 2.5 g/g is regarded as a good-quality protein.[22]

In addition to having a high PER, cow's milk whey protein contains a higher percentage of essential amino acids per gram of protein compared to most plant and other animal protein sources (refer to Figure 3).[18] Essential amino acids are critical for building proteins, as they are not made within the body and must be obtained from the diet. A measurement used by the World Health Organization to evaluate the amount of essential amino acids available for protein synthesis after digestion is the protein digestibility corrected amino acid score.[51] A protein's score is based on the requirement of essential amino acids needed by a preschool-aged child for growth. Whey has a "perfect" score of 1.0, meaning that the concentration of essential amino acids remaining after whey is digested fulfil the daily essential amino acid requirements of a growing 3-5 year old child.[51,52]

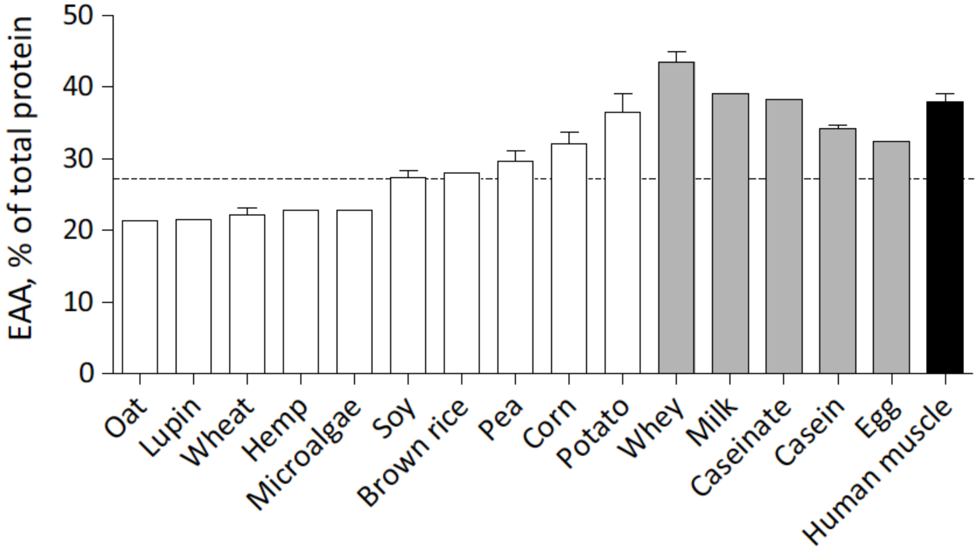

Figure 3: The Percentage of Essential Amino Acids in Cow's Whey Protein compared to Other Plant and Animal Protein Sources and Human Skeletal Muscle[18]

*Dotted line represents the percentage of protein that should be consumed in the form of essential amino acids, recommended by the World Health Organization

A particular essential amino acid found in high concentrations in whey protein is the branched-chain amino acid, leucine. Whey protein contains 8.6 grams of leucine per 100 grams of unfiltered powdered whey, whereas the concentrations of other essential amino acids range from 2-7 grams per 100 grams of unfiltered powdered whey.[18] The recommended intake of leucine for a child one year of age is 0.063 grams per kilogram of body weight per day and decreases as the child ages as rates of growth decline.[53] Therefore, an average one-year old child weighing 9 kilograms would require 0.73 grams of leucine per day. Because 2.7 grams of leucine are found in 32 grams of unfiltered powdered whey, this child would need to consume 1.5 tablespoons of powdered whey to meet their daily leucine requirement. This makes whey an acceptable source of leucine, especially for a growing child.[18] Although leucine is found in high concentrations in whey, it is important to remember that all essential amino acids are needed for protein synthesis to occur.

Due to the high concentration of essential and branch-chained amino acids in whey, this type of protein has been used to support the nutritional rehabilitation and physical growth of malnourished infants and children in low-income countries.[54,55] Infants and children who consumed whey protein that was added to ready-to-eat supplementary foods, in comparison to infants and children who consumed soy protein, had better growth markers including increased mean weight-for-height z-scores and greater gains in mid upper arm circumferences.[54] Though these gains in weight and mid upper arm circumference were most likely due to immediate increases in fat deposits, this research suggests that whey is an appropriate protein source to improve the recovery rates of malnourished children living in resource-poor countries. Unfortunately, research using whey protein to support the nutritional rehabilitation of malnourished adults living in low-resource countries has not been established; however, whey protein has shown to stimulate muscle protein synthesis in adults at rest better than casein or soy protein.[56-58] Retaining and synthesizing new muscle while recovering from malnutrition is important, as low muscle mass is related to longer recovery times and increased morbidity and mortality.[59]

Potential Problems from Early Infant Consumption of Non-Human Mammalian Milk

Although animal milks provide beneficial nutrients for growing children, infants who consume animal milks, aside from modified animal milks found in approved infant formulas, before one year of age can experience iron deficiency anemia, dehydration, and megaloblastic anemia.[60] Iron deficiency anemia; or a decreased production of red blood cells that are small in size and cannot efficiently carry oxygen throughout the body; is attributed to two factors. First, animal milks do not contain sufficient amounts of bioavailable iron to support infant growth and the expansion of red blood cell volume. Though human breast milk and animal milks contain low concentrations of iron, infants absorb 50% of the iron found in breast milk and only 10% of the iron found in animal milks.[60] If exclusively breast fed, 1 milligram of supplemental iron for each kilogram of body weight should be given to infants each day, beginning at 4 months of age, in addition to the iron that they would receive from breast milk to meet the estimated iron requirements for physical growth and cognitive development.[61] In contrast, infants who consume animal milks would require more iron supplementation and infants who consume approved infant formulas require no additional iron as iron is added to these products. The second factor related to iron deficiency anemia is blood loss from the gastrointestinal tract due to irritation and the inability of the infant to easily digest animal milks.[60] The combination of low iron bioavailability and gastrointestinal bleeding, leading to iron-deficiency anemia, is concerning, as lower numbers of red blood cells reduces the amount of oxygen circulating throughout the body.

Dehydration, another potential side effect of an infant consuming non-human mammalian milk, stems from the high protein and mineral content found in animal milks, or the renal solute load.[60] Renal solute load refers to the dietary solutes, specifically nitrogen, sodium, potassium, chloride, and phosphorus, that must be excreted by the kidneys.[61] Because animal milks contain higher concentrations of dietary solutes than breast milk and infant formulas, infants who consume animal milks before one year of age produce urine with a high osmolarity. Producing urine with a high osmolarity places the immature kidneys of an infant under significant stress and becomes dangerous during times of low fluid intake, as it quickly leads to dehydration.[60] It is recommended that infants do not consume more than 3.4 grams of protein per 100 calories to maintain a safe and reduced renal solute load.[62] To put this into perspective, breast milk contains an average of 1.5 grams of protein per 100 calories,[63,64] cow's milk contains 5.2 grams of protein per 100 calories[65] and standard infant formula provides 2.1-2.2 grams of protein per 100 calories.[66]

Megaloblastic anemia, or the production of large, immature red blood cells, is a specific concern when infants consume non-modified goat's milk.[67,68] To produce mature, fully functional red blood cells that can deliver oxygen throughout the body, the micronutrient folate is needed. Folate plays a role in the production of purines, or the bases adenine and guanine, for DNA synthesis. When insufficient amounts of folate are consumed, DNA synthesis is impaired, and thus, red blood cells cannot divide or mature.[69] Goat's milk contains 6 micrograms of folate per liter of milk, whereas human breast milk contains 45 to 50 micrograms of folate per liter of milk.[67] Because infants require 65 to 80 micrograms of folate each day, depending on age[70], non-modified goat's milk is not an acceptable source of nutrition for infants.

As can be seen, animal milks and whey protein derived from animal milks, without modification, are not suitable for infants. However, animal milks and whey protein contain high concentrations of essential amino acids and nutrients that are appropriate to support the growth and development of malnourished children and rehabilitate malnourished adults. Whey powder though that is used as a supplemental source of dietary protein is often derived from cow's milk. Dairy cattle in Lao PDR are not common, and therefore, a different source of milk is needed to derive a local, sustainable source of whey protein for use in Lao PDR.

Dairy Cattle in Lao PDR

Cattle found throughout Lao PDR are small in size, are raised for meat, and are typically not used for milking. Due to temperatures in Lao PDR ranging from 29-45°C (84-113°F) and an average humidity of 75% throughout the year, very few large cattle that would be used for milking live in the country.[71] Heat and humidity combined, expressed as a heat index, above 20°C cause dairy cows to experience heat stress.[72] Significant heat stress occurs at or above 27°C and leads to loss of appetite, decreased milk production, and susceptibility to sickness and disease.[72] The microflora found in the cow's digestive system make it vulnerable to heat stress by producing high amounts of heat needed to break down fibrous materials.[72] Larger cows are more prone to heat stress than smaller cows, which is the reason why smaller cows tend to be raised in Lao PDR.[72] Without a proper facility to create livable conditions for dairy cattle, the production of cow's milk is challenging.

Dairy Cattle Milk Products

Dairy cattle milk-based products are available in Lao PDR for purchase but are not common in rural areas. Milk and yogurt sold in grocery markets or convenience stores are primarily imported from surrounding countries. The majority of milk is imported from Thailand, and Lao PDR receives 23% of Thailand's total exported cow's milk.[73] Overall, cow's milk is only available in limited quantities and locations throughout Lao PDR, making it a difficult source of protein to acquire in the diet. Water buffalo, however, are a common livestock animal found in Lao PDR that can produce milk.

Water Buffalo in Asia

Water buffalo are local to Asia and were domesticated over 5,000 years ago in India and Pakistan.[73] Approximately 97% of the world's water buffalo population is located in Asia, with the largest herds of buffalo in India, Pakistan, and China.[12] Of the 171 million water buffalo in Asia, almost 75% live in South Asia. This includes all water buffalo living in Afghanistan, Bangladesh, Bhutan, India, Maldives, Sri Lanka, Pakistan, and Nepal. Approximately 8.4% of all water buffalo live in Southeast Asia,[74] and Lao PDR has 750,000 of these water buffalo within the country,[75] or 0.639% of the world's total water buffalo population.[74]

Water Buffalo Breeds

There are two general types of water buffalo found throughout the world: river and swamp water buffalo. River water buffalo make up roughly 70% of the world's water buffalo population and are the principle source of water buffalo milk in Asia.[13] A common breed of river water buffalo used specifically for milk production is the Murrah. Murrahs are larger than swamp buffalo and are identified by their dark color and curved horns.[76]

River water buffalo can be found in Southeast Asia; however, swamp water buffalo are more common. Swamp water buffalo are identified by their smaller size and straight horns. Historically, swamp water buffalo were used as draught power to till and plant rice fields. Today, electric motor tillers are used to prepare and plant fields, and swamp water buffalo are used to provide manure for fertilizer and can be sold to produce edible beef.[12]

Although the traditional breeds of river and swamp water buffalo are used for different purposes, the two can be cross-bred to increase the animal's milk supply.[77] This has been accomplished in China, India, and Lao PDR.[11,75,77] Currently, Murrah river water buffalo are being crossbred with general swamp water buffalo to increase the swamp water buffalo milk yield and gene pool. In turn, this will expand the use of water buffalo milk throughout Asia.[75,77]

Breeding Water Buffalo

Water buffalo can safely breed at three years of age and have a ten-month gestational period.[75] Often, pregnancy is accomplished through artificial insemination, but also occurs naturally. In Lao PDR, natural water buffalo pregnancy rates are highest during the cooler months of October through March. However, breeding at this time of year can be problematic. Calves that are born ten months later, between the months of August and January, wean at four months of age between December and May and transition to grazing. The transition occurs during the dry season when grasslands and pastures are scarce and there is a limited source of feed. This contributes to a high calf death rate in Lao PDR.[75]

Water Buffalo Milk and Production in Asia

Water buffalo are a common source for milk throughout Asia. India and Pakistan, in particular, produce more milk from water buffalo than dairy cows.[12] Table 1 shows the production of both water buffalo and cow's milk from the largest water buffalo herds in the world.[12]

Table 1: Water Buffalo and Cow's Milk Production in India, Pakistan, and China[12]

Country	Water Buffalo Milk*	Cow's Milk*	Total Milk Produced*
India	67.7	59.4	127
Pakistan	16.3	13.4	29.7
China	3.1	36.8	39.9

*Millions of tons
Data collected in 2010

Water buffalo in Lao PDR produce milk for an average of six months after birthing a calf.[75] Other countries throughout Asia report water buffalo lactation periods up to 325 days, or eleven months, out of the year.[11] Water buffalo milk collection, for commercial purposes, often begins directly after the birth of a calf. However, in Luang Prabang, Lao PDR, milking usually begins three weeks after a buffalo gives birth.[75] This ensures that the buffalo calf receives the milk's colostrum and a sufficient milk supply during the early-post-partum period.[75] Milking is done using a goat or cow cup and an automated milk pump system. Goat cups are used when buffalo are new to milking. At first, water buffalo teats are small and can be injured using a cow cup. As water buffalo are milked over time, their teats stretch and cow cups can be used.[75]

Daily milk production varies depending on the water buffalo breed. For example, swamp buffalo produces 1-2 liters of milk per day, whereas river buffalo, such as Murrah, produces on average 6.5 liters of milk per day.[11] In China, it has been reported that crossbred Murrah/swamp water buffalo produce 4-4.5 liters of milk per day,[11] which is an improved milk yield compared to purebred swamp water buffalo. The Laos Buffalo Dairy located in Luang Prabang, Lao PDR has successfully bred 50 Murrah and Lao PDR swamp water buffalo. However, no information on crossbreed milk production has been established.[75] On average, water buffalo can reproduce every other year and be milked for up to 20 years of age.[13] Although milking water buffalo is a common practice throughout Asia, it is a fairly new concept to many farmers and families in Lao PDR.[13]

Water Buffalo Milk Nutrient Profile

Compared to dairy cow's milk, water buffalo milk is higher in energy, fat, and protein.[10] The fat content of water buffalo milk ranges from 6-8%, which is up to 50% higher than cow's milk.[19,78,79] Compared to other mammalian milks, water buffalo milk contains higher concentrations of lactose, total solids, and carbohydrates.[19] This is attributed to water buffalo milk's lower water content.[19]

For the same reason, water buffalo milk is also "high" in calcium, iron, phosphorus, B-12, potassium, and magnesium compared to other mammal milks. Specifically, the mineral concentrations of calcium, phosphorus, and magnesium are considered "high" because they can provide sufficient amounts that meet the recommended human dietary intake for these nutrients when 8-13 ounces of liquid milk is consumed (Table 2).[10,19,79-81] Although present in trace amounts, water buffalo milk also contains vitamin E, folic acid, and vitamin D.[80] Water buffalo milk nutrient profiles change and are highly dependent on the month, season, period of lactation, and number of previous lactation cycles.[79,82]

Table 2: Nutrient Concentrations of Local Egyptian Water Buffalo, Cow, Camel, Goat, and Human Milks [19]

Nutrient*	Water Buffalo	Cow	Camel	Goat	Human
Total Energy (kcal)	105.1 ± 1.2	71.1 ± 1.05	70.1 ± 0.91	67.4 ± 0.84	74.1 ± 0.57
Total Fat (g)	7.52 ± 0.05	4.14 ± 0.03	4.2 ± 0.11	4.04 ± 0.05	4.17 ± 0.06
Protein (g)	4.02 ± 0.05	3.48 ± 0.03	3.27 ± 0.06	3.32 ±0.03	1.11 ± 0.04
Carbohydrate (g)	5.33 ± 0.05	4.98 ± 0.02	4.67 ± 0.10	4.44 ± 0.02	8.04 ± 0.06
Lactose (g)	5.02 ± 0.03	4.70 ± 0.03	4.31 ± 0.10	4.27 ± 0.02	7.12 ± 0.07
% Lactose†	94.32 ± 0.32	94.46 ± 0.23	92.37 ± 0.53	96.26 ± .13	88.57 ± 0.54
Ash (g)†	0.80 ± 0.00	0.71 ± 0.00	0.75 ± 0.01	0.83 ±0.00	0.21 ± 0.003
Total Solids†	17.65 ± 0.10	13.30 ± 0.13	12.95 ± 0.13	12.62 ± 0.06	13.53 ± 0.08
Moisture (g)	82.33 ±0.10	86.70 ±0.13	87.05 ± 0.06	87.38 ± 0.06	86.47 ± 0.08
Ca (mg)	163.2 ± 4.56	119.9 ± 0.69	111.4 ± 4.36	130.3 ± 2.26	32.4 ± 0.70
P (mg)	111.4 ± 2.61	95.0 ± 0.72	81.2 ± 3.08	110.2 ± 1.61	14.0 ± 0.24
Fe (mg)	0.135 ± 0.007	0.07 ± 0.02	0.23 ± 0.01	0.06 ± 0.00	0.053 ± 0.004
Zn (mg)	0.24 ± 0.008	0.38 ± 0.00	0.51 ± 0.015	0.32 ± 0.03	0.165 ± 0.02
Na (mg)	51.61 ± 0.66	49.67 ± 0.70	57.84 ± 1.22	50.33 ± 0.77	16.03 ± 0.31
K (mg)	167.2 ± 3.16	147.0 ± 1.55	156.3 ± 2.85	201.6 ± 1.90	51.8 ±0.69
Mg (mg)	29.56 ± 0.79	13.42 ± 0.24	6.70 ± 0.14	13.87 ± 0.11	3.43 ± 0.12

***Per 100 grams of liquid milk**
***Mean ± Standard Error**
†**Total Solids:** All non-water components in milk, including protein, ash, fat, and lactose
% Lactose: Total percentage of carbohydrate that is composed of lactose
Ash: Concentration of all minerals

Water Buffalo in Lao PDR

Water buffalo are a staple farming animal in Lao PDR due to their long, 25 year lifespan; resistance to parasites; and ability to thrive in hot, tropical climates.[10,11,83] Although a young, local Lao PDR water buffalo can cost up to $1,200 United States dollars, which is similar to the average yearly salary in Lao of ₭10,620,00 Kip**,**[84] most agricultural families own 2-3 water buffalo.[75] The large value that these animals hold turn water buffalo into a living “savings account” that families can sell in times of economic crisis.[13,85]

Water Buffalo Programs in Lao PDR

In 2016, the Laos Water Buffalo Dairy located in Luang Prabang, Lao PDR was established, and since, has been working towards improving the care of water buffalo throughout Lao PDR. The farm has created a breeding program to increase calf survival rates, provides vaccinations to the buffalo, and milks the buffalo to produce cheese and other dairy products.[75] The dairy demonstrates to local farmers how to properly care for and raise water buffalo to create a healthy buffalo population that can be utilized in the country’s favor. As a part of this initiative, the dairy “rents” pregnant water buffalo from local farmers to provide regular incomes to families. During the “rental period”, water buffalo are brought to the dairy, vaccinated, are incorporated into the dairy herd, and are kept safe and healthy.[75]

Introducing farmers to the concept of milking their buffalo is important, as water buffalo milk could be used a potential source of energy, protein, and other essential nutrients for human consumption. In addition, the whey fraction of water buffalo milk derived from the cheese-making process may contain beneficial protein that would support growing children and adults, especially those who are malnourished.

Whey Production During the Cheese-Making Process

During the cheese-making process, the two major animal milk proteins, casein and whey, separate to form solid cheese curd and a liquid by-product, respectively. Casein milk protein coagulates to become cheese curd after milk is heated to a high temperature, and enzymes (such as rennet) or acids (typically citric acid) are added.[17,22,86] As casein coagulates to become cheese curd, a liquid byproduct simultaneously forms. This remaining liquid contains the other major milk protein, whey. Unfiltered liquid whey that has separated from the cheese curd is approximately 87% water and includes 6-10 grams of protein and 46-52 grams of lactose per liter of fluid.[17] In addition, whey contains lipids, acid, minerals, water soluble vitamins, and small cheese particles that separate from casein during cheese curd production.[22]

Categories of Liquid Whey

The liquid whey by-product from the cheese-making process is either classified as “sweet” or “acid” whey depending on the type of cheese made.

Sweet (non-acidic) whey is derived after making hard cheeses that use the enzyme, rennet, to separate milk into the solid cheese curds and the liquid whey. Because enzymes are used in this particular cheese-making process instead of acid, the pH of sweet whey is higher.[22]

Acid whey is formed after making yogurt or soft cheeses such as mozzarella, ricotta, feta, cottage cheese, or cream cheese. The whey derived from this process is called “acid” because of the low pH that occurs as a result of the formation process. Although enzymes such as rennet can be used to produce acid whey, a higher amount of acid, often lactic acid or citric acid, is added to heated milk during the cheese-making process.[22]

In general, leftover liquid acid and sweet whey are strong environmental pollutants due to their high content of lactose, proteins, and minerals, known as organic matter, and therefore, are difficult to dispose of.[87] Often, cheese-making facilities feed their whey “waste product” to animals as an option for disposal.[87] However, liquid whey can also be dried and utilized as a source of protein for human consumption. To use liquid whey as a source of protein and other nutrients for humans, it must be pasteurized, filtered, and dried to create a safe, shelf-stable powder.

Whey Protein Processing

Liquid Whey Pasteurization

Liquid whey must be pasteurized before processing to destroy harmful bacteria and deactivate enzymes used to separate casein from whey. Commercial pasteurization requires that milk or whey solutions are heated to 72°C or higher for at least 15 seconds.[22] After pasteurization, liquid whey is either immediately dried or filtered prior to drying.

Liquid Whey Filtration

Filtering liquid whey increases the whey protein concentration while removing lactose, excess water, and other non-protein components such as fats and minerals.[22] The first step in the filtration process is ultrafiltration. In ultrafiltration, permeable membranes are used to separate molecules in liquid whey based on particle size. Smaller molecules of lactose, water, and soluble minerals pass through the filter and separate as leftover dairy solids known as the permeate; while whey protein, fatty acids, and insoluble salts that cannot pass through the filter are retained and known as the retentate.[22,88] The time required for ultrafiltration depends on the desired volume of retentate. A smaller end volume of retentate contains a higher concentration of whey particles and requires a longer filtration time.[89] Ultrafiltration is carried out at temperatures below 55°C to reduce the risk of denaturing the whey protein. If whey processing stops after ultrafiltration, a 35-50% whey protein concentrate results. This solution can be dehydrated and powdered into a protein supplement.[17,22]

To produce a higher concentration whey protein while dramatically reducing its lactose and mineral salt concentrations, whey retentate can undergo diafiltration. In diafiltration, water is added to the whey retentate to decreases its viscosity and wash out lactose and mineral particles that were not removed during ultrafiltration, yielding a 50-80% whey protein concentrate.[22,89] Higher protein quantities are best achieved through multiple low-volume cycles of diafiltration following a long ultrafiltration period that results in a highly concentrated whey retentate.[89] The final whey protein concentrate typically contains 65-80 grams of protein and 4-21 grams of lactose per liter.[17]

Whey protein concentrate can be further processed through microfiltration or ion exchange to increase protein concentration up to 90% and remove almost all lactose, fat, and minerals. This procedure produces whey protein isolate, which is more expensive to produce and results in a smaller volume of product after removing all non-whey particles.[17,22]

Although filtering liquid whey is not necessary, unfiltered whey can be problematic during the drying process. Excess lactose that is not filtered causes protein aggregation, resulting in the formation of larger, undesired dried whey particles that are difficult to return to a smooth solution when rehydrated.[90] In addition, unfiltered whey has a shorter shelf life due to the minerals and other non-protein components, such as fatty acids, degrading in the final dried product. Specifically, fatty acids are susceptible to oxidation which results in the rancidity of the whey protein.[22,91] Therefore, filtering liquid whey before drying is preferred to prevent whey processing complications.

Liquid Whey Drying

To inhibit microbial growth, prolong shelf life, preserve nutritional quality, and reduce costs associated with packaging and shipping, liquid whey must be dried. The traditional drying process involves the use of hot, dry, moving air to evaporate moisture. However, drying can also occur through lyophilization, or freeze-drying: the process of heat and low pressure instantly changing ice crystals into vapor. If the traditional drying method is used after producing filtered, concentrated liquid whey, either a spray dryer or drum roller is used.

A spray drying system consists of a feed pump, an atomizer or “spray nozzle”, a large drying chamber, and an air heating system as shown in Figure 4.[92] Feed pumps are responsible for transporting liquid whey to the top of the drying chamber where the air heating system has filled the chamber with hot air. Whey enters the drying chamber as mist through the automizer as hot air circulates and immediately dehydrates the liquid. During the evaporation process, the overall temperature of the whey particles remains lower than the air distributed in the drying chamber, as the lower particle temperature minimizes the denaturation of whey proteins. Drying chambers alone change liquid whey into a wet powder with a 10-14% moisture content. Whey powder must contain no more than 5% moisture to reduce its water activity, minimize microbial growth, and be shelf-stable. Therefore, wet whey powder is further dried after being processed in the spray dryer through a fluidized bed.[93]

Processing through a fluidized bed forces semi-dried whey particles to fall to the bottom of the drying chamber onto a vibrating porous plate. Cooler air blows through pores in the plate to induce whey "fluidization". The air velocity of this process must be precise; an air velocity too high will send particles out into the gas stream and away from drying, and an air velocity too low will not induce correct fluidization. The combination of accurate air velocity and constant vibration stops whey particle clusters from forming and creates small, individual whey particles with an acceptable moisture content of 3-5%. The completed process is considered "two-staged" drying. Whey particles are then separated from the air used for drying through a cyclone and collected as a dried powder.[17,92,93]

The two-staged drying process using a spray drier can produce ideal whey protein powders. This is because small, equal-sized particles that can easily dissolve in water are created. Additionally, two-staged drying avoids denaturing whey protein and minimizes whey protein heat damage.[90]

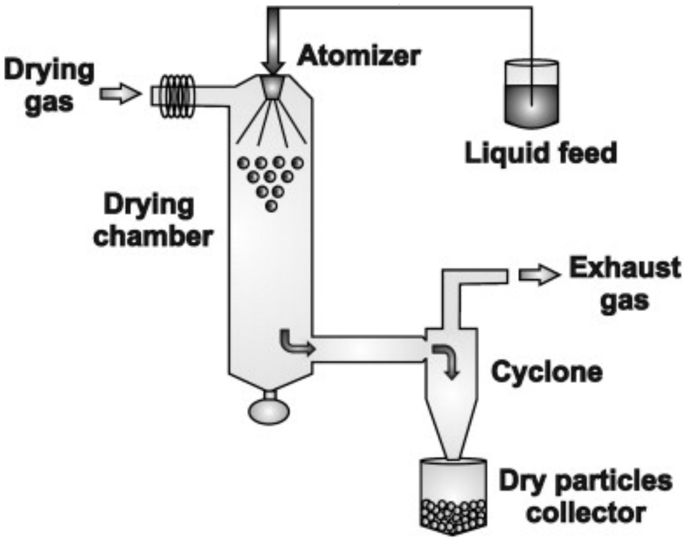

Figure 4: Spray Dryer Used to Dry Liquid Whey[92]

The second traditional method used to dry whey protein is a drum roller, as illustrated in Figure 5.[78] A drum roller consists of large rollers heated with steam inside a large cylindrical chamber. As the rollers rotate, liquid whey is added and dried on the rollers themselves by hot steam. Dried whey is then scraped off roller surfaces by a shear. Constant scraping of the rollers inhibits protein aggregates from forming and results in a high quality powdered product.[90] Although drum roller dryers are effective in quickly drying liquid whey, this method does not produce the same small, round particles like a spray dryer. Particles are instead rolled flat into flakes and then broken up into a powder.[90,94]

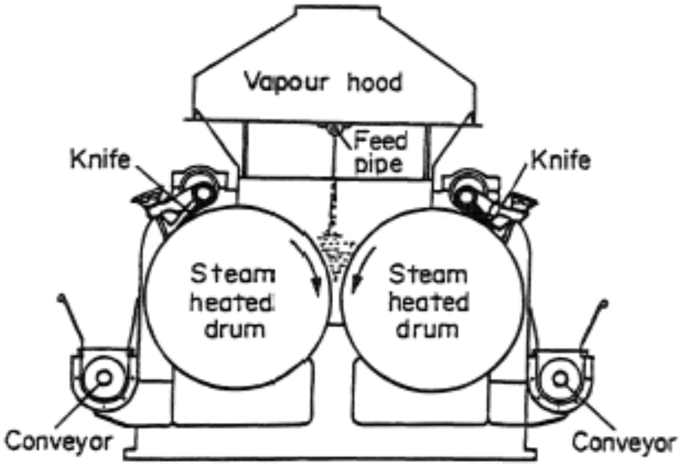

Figure 5: Drum Roller Used to Dry Liquid Whey[78]

Freeze drying is a unique, high energy drying method typically not used to preserve milk products as shown in Figure 6.[81] The process, known as lyophilization, begins by placing products on a tray and then into a drying chamber held at -40 to -60°C. Low temperatures turn all food moisture into ice crystals. Once frozen, a vacuum pump reduces pressure inside the drying chamber while a heating system raises the chamber's temperature. Heat and low pressure combined cause all ice crystals to sublimate, or transition directly from a solid state into a gaseous state. Vapor is collected in an ice condenser located outside of the drying chamber, converted back into ice, and removed through a draining system.[17] Total drying time ranges from 24-36 hours for small home batches or up to 100 hours for commercial drying.[95,96] Home freeze dryers can dehydrate a little over 2,000 liters of liquid per year, whereas some commercial freeze-dryers can dehydrate over 5,000 liters of product per batch.[95-97]

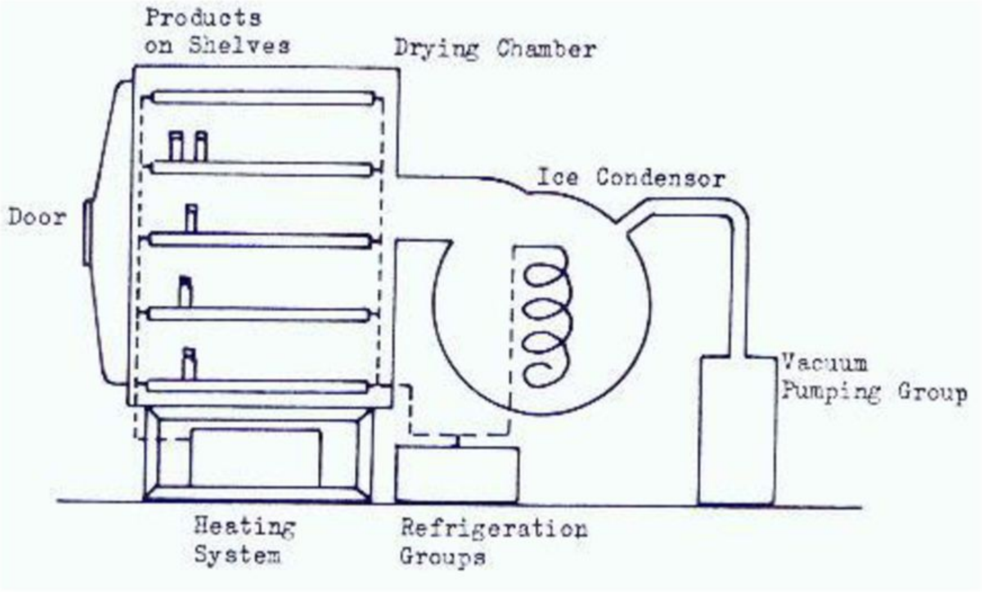

Figure 6: Freeze-Dryer Used to Dry Liquid Whey[81]

Because the freeze-drying process uses low temperatures to remove moisture in foods, traits found in heat-dried products such as browning, protein denaturation, surface/case hardening, and off flavors, do not occur. Ice crystal formation and removal also create pores in dehydrated finished products. This is advantageous in that water can easily rehydrate products to their original form. Freeze-dried foods are favorable, but expensive to produce, as higher energy demands from the low freezing temperatures, re-heating of the drying chamber, and the long drying time cycle increase costs.[98]

Common Problems Associated with Whey Protein Processing

Turning liquid whey into a useable, shelf-stable source of protein is ideal, but can be difficult. One common problem that occurs during whey processing is heat damage created by drying whey at too high of a temperature. Heat damage to whey particles denatures the protein, and as a result, large aggregates form and particle sizes increase. Increased particle sizes require a longer drying time to reduce whey moisture contents to less than 5%, and the longer drying time results in a poor-quality whey product with heat damage.[90,93] Final powdered products with heat damage will be gritty, taste chalky, have reduced solubility, and will release gels that cause whey particles to form as large globules. In comparison, finished powdered products with small, uniform particles without heat damage or denatured protein produce smooth, creamy whey powders.[90]

Unfiltered lactose is also problematic during the dehydration process. When lactose is not filtered from the whey solution before dehydration, it has a strong influence on protein denaturing and particle size. Although lactose slows down and protects against protein denaturation, higher amounts of lactose cause greater protein aggregation and overall larger, undesired whey particles.[90]

The type of liquid whey used to create a final powdered whey product significantly affects whey processing as well. Acid whey is difficult to dehydrate and powder due to its high lactic acid content that causes whey protein to agglomerate and form lumps in the drying equipment. It is possible to neutralize the pH of acid whey with "additives", such as defatted milk, to facilitate the drying process. However, the preferred method to neutralize the whey solution is to remove the lactic acid by filtration before dehydrating. This requires more time and effort.[22] In comparison, sweet whey is commonly filtered to yield commercial whey protein concentrate or whey protein isolate powders. The physical and chemical properties of sweet whey make it easier to work with when making powdered whey, and therefore, it is favorable.[22]

Overall, processing liquid whey into a desirable source of protein is a precise and delicate science.

Whey Protein Shelf Life

Final whey protein powders sealed in airtight, non-specialized packaging and stored, unopened, at a room temperature of 20°C have a shelf life of up to 12 months.[22,91] The shelf life changes to 9 months when whey is exposed to high humidity levels and temperatures of 30-35°C. The reduced shelf life of whey protein is attributed to the breakdown of the essential amino acid lysine. Due to lysine's chemical structure, leftover lactose remaining in dehydrated whey reacts with the amino group on the end of lysine's side chain. This reaction causes Maillard browning to occur and bound water within whey protein to be released. The release of bound water increases moisture content and water activity in powdered whey; however, the excess moisture is not enough to cause new bacterial growth. Lipid oxidation can also occur and decrease the quality of whey protein in high whey protein concentrates (80% whey protein concentrate). This type of decomposition is not significant until after the 9-month shelf life expectancy.[91]

Gaps in the Research

Few studies address the nutritional composition of water buffalo milk and no published research addresses the nutrient profile of Lao PDR water buffalo milk and its acid whey fraction derived from the soft cheese-making process. The majority of whey research, and exclusively the whey research presented here, describes how cow's milk whey is processed to a powdered protein supplement. Water buffalo milk whey may have similar or completely different properties as cow’s milk whey. To determine if Lao PDR water buffalo milk and its liquid and reconstituted powdered acid whey fraction contain adequate amounts of energy, protein, and other nutrients to use as a powdered supplement, nutrient analyses of these products are needed.

CHAPTER 3

Methods

General Design

Raw unpasteurized/non-homogenized water buffalo milk and acid whey samples were collected from pooled samples at the Laos Buffalo Dairy located in Luang Prabang, Lao PDR. Because the Laos Buffalo Dairy only produces acid whey from the cheese-making process, sweet whey was not available for analysis. Milk samples and two of the three acid whey samples were collected on the same day. The third acid whey sample was collected on a different day due to the cheese-making production schedule. All samples were refrigerated and taken to the Institute of Nutrition at Mahidol University in Bangkok, Thailand for nutrient analyses. The average energy and nutrient concentrations of water buffalo milk were compared to reference data on raw, unpasteurized cow's milk as published by Soliman[19] and to reference data on water buffalo milk as published by Soliman[19], Wong[99], Han et al.[79], and Khan et al.[100] The Soliman source was used because there is no known reference to us on raw, unpasteurized/non-homogenized cow's milk in the published literature. Mean nutrient concentrations of liquid and powdered water buffalo milk acid whey were compared to reference data on liquid and powdered acid whey derived from cow's milk included in the USDA FoodData Central nutrient database.[20,21] The protein concentration of acid whey samples were compared to the United States Dairy Export Council standards for acid whey derived from cow's milk to determine the suitability of water buffalo milk acid whey as a protein supplement. Due to the limited number of water buffalo milk and acid whey samples, descriptive analyses were performed to summarize the data and compare these to reference values, only.

Water Buffalo Milk and Whey Sample Collection

Water buffalo milk and acid whey samples were collected from pooled milk and whey derived from dairy-raised and locally-rented Murrah and Lao PDR Swamp water buffalo eating a diet consisting of 26 kilograms African Napier grass and 4 kilograms mixed cassava, corn, and soybean for a total of 30 kilograms of dry feed per day.[75] Water buffalo raised at the Laos Buffalo Dairy are milked twice a day; once between 6-8 A.M. and again at 5 P.M., if able. Water buffalo that are rented from local farmers are milked once per day and are returned to their calves for feeding.[75]

Four buffalo are milked at a time. A bleach solution is used to sanitize the buffalo teats and a goat or cow cup is attached to each teat and then to an automated milk pump system. Milk from each animal is collected in individual milking tanks and directly transported to cold milk-pooling containers. After milking, filled pooling containers are placed into a deep freezer to lower the milk temperature to a safe, non-frozen state until pasteurization occurs. For pasteurization, milk in the chilled pooling containers is transported to the kitchen to undergo a slow, low temperature pasteurization that kills harmful bacteria while preserving beneficial bacteria. As a part of this process, milk is slowly heated to 63°C for thirty minutes and then cooled to 4.5°C. It is essential that the milk is never brought to a boil, as cheese cannot form using boiled milk.[75]

Three, 1-liter water buffalo milk samples were collected from fresh pooled milk, prior to pasteurization, from water buffalo milked that same morning. Milk samples were stored in sterile bags used for dairy cow research and were collected from a kitchen refrigerator set at 2°C**.** Water buffalo milk and acid whey samples were transported by air, on ice, in a cooler (YETI Model: Roadie 20, Austin, Texas, USA) and received by the Institute of Nutrition, Mahidol University, Bangkok, Thailand within 50 hours of collecting the milk samples from the kitchen refrigerator.

Liquid acid whey samples were derived from single batches of feta, mozzarella and ricotta cheese made from previously pasteurized pooled water buffalo milk.

Feta Cheese Making Process and Whey Sample Collection

Feta cheese is produced by adding a MM100 mesophilic starter culture to chilled pasteurized water buffalo milk that is slowly heated to 30-32°C. Rennet, an enzyme that is derived from animals and is used to separate milk into solid cheese curds and liquid whey, is added to the hot milk and left to rest until the cheese curd and whey separate. Once the cheese curd forms, it is cut and stirred multiple times every ten minutes to further release the whey from the cheese curd. The feta cheese curd and whey mixture are separated using a colander.[75] A 1-liter sample of feta liquid acid whey was collected into a plastic milk container and was stored in a freezer set at -18°C until transported to Mahidol University for analyses.[75]

Mozzarella Cheese Making Process and Whey Sample Collection

Mozzarella cheese is produced by adding citric acid to chilled pasteurized water buffalo milk that is slowly heated to 32°C. Rennet is then added to the hot milk and left to rest for approximately ten minutes, or until cheese curd forms and whey separates from the curd. After cheese curd has formed, liquid acid whey is separated using a colander. The cheese curd retained from the colander is stored in a refrigerator set to 2°C.[75] A 1-liter sample of liquid mozzarella acid whey was collected into a sterile milk research bag and stored at 2°C until transported to Mahidol University for analyses. The remainder of the liquid acid whey was used to make ricotta cheese.

Ricotta Cheese Making Process and Whey Sample Collection

To make ricotta cheese, liquid whey derived from the mozzarella cheese-making process is heated until almost boiling. Apple cider vinegar is then added to form a secondary cheese curd and a residual whey product. The ricotta cheese curd and whey mixture is separated using a colander.[75] A 1-liter sample of liquid ricotta acid whey was collected into a plastic milk container and stored at 2°C until transported to Mahidol University for analyses.

Composite Analyses of Milk and Liquid Acid Whey Samples

Research staff at the Institute of Nutrition at Mahidol University, Bangkok, Thailand received the milk and acid whey samples for analyses within 50 hours of shipment from the Laos Buffalo Dairy. One of the three milk samples was analyzed in duplicate for total energy, crude fat, crude protein, total carbohydrate, ash, moisture, calcium, and lactose concentrations. The other two samples were analyzed in duplicate for total energy, crude fat, crude protein, total carbohydrate, ash, and moisture, only.

One-half liter of each acid whey sample was analyzed in its original liquid form. The other half liter of each acid whey sample was lyophilized to form a powder and then reconstituted with water before undergoing analyses. Each acid whey sample was analyzed in duplicate for total energy, crude fat, crude protein, total carbohydrate, ash, and moisture concentrations.

Compositional Analyses

Crude Fat Analysis

Crude fat content of each sample was determined following automated acid hydrolysis and solvent extraction using Soxtec™ technology.[101] Acid hydrolysis of fatty acids and lipid-carbohydrate bonds is achieved using hydrochloric acid. The hydrolyzed fatty acids are then dissolved in a solvent after which the solvent is removed using evaporative extraction. The remaining fat is weighed to determine the crude fat concentration of the original sample.[102,103]

Crude Protein Analysis

Crude protein content of each sample was measured using the Kjeldahl method and combustion.[101] In the Kjeldahl method, protein is digested in sulfuric acid with the addition of a catalyst, such as copper or selenium. Organic nitrogen is then converted into ammonia under acidic conditions. Ammonia is distilled into boric acid, titrated with hydrochloric acid, and then measured as nitrogen. The measured amount of nitrogen is used to calculate the crude protein concentration using a conversion factor of 6.25 such that nitrogen (g) x 6.25 = crude protein (g).[104]

In the combustion method, samples are combusted with oxygen. All gasses containing nitrogen oxides are collected in a tank and pressurized. Nitrogen oxide gasses are reduced to nitrogen and passed through a tube containing magnesium perchlorate and sodium hydroxide. This removes water and carbon dioxide from the gas. The remaining nitrogen is measured and converted to grams of protein using a conversion factor of 6.25.[104,105]

Total Carbohydrate Calculation

Total carbohydrate in grams was determined as the difference in weight of 100 grams of milk and the measured weight of each constituent component: protein, fat, ash, and moisture such that total carbohydrate (g) = 100 g milk – [total protein (g) + total fat (g) + ash (g) + moisture (g)].[101]

Total Energy Content

Energy contents of each 100 gram sample (kcal/100g) was determined by the amount, in grams, of each macronutrient; carbohydrate, fat, and protein. Total grams of carbohydrate, fat, and protein were converted to their energy equivalents by multiplying each gram amount by 4, 9, and 4 kcals/g, respectively, and adding these together to estimate the total energy content of each sample [Total energy (kcal/100g milk or acid whey) =(carbohydrate (g) x 4 kcal/g) + (fat (g) x 9 kcal/g) + (protein (g) x 4 kcal/g)].[101]

Lactose Analysis

Lactose concentrations were measured with a refractive index detector following high pressure liquid chromatography (HPLC).[101] Briefly, HPLC involves two main stages: mobile and stationary. During the mobile stage, a diluted sample of milk is injected into a moving liquid solvent powered by a high-pressured pump. The solvent carries the sample to an HPLC column where the stationary stage begins. Inside the column, the constituents of the sample are separated from the solvent through pressure elicited in the column. The separated constituents then pass through a detector to be quantified based on their fractional elution times. Results are converted back into the original constituent concentrations using a standard curve.[106]

Moisture and Ash Analysis

Moisture and Ash contents were determined using two gravimetric techniques.[101] First, liquid samples were dried in a forced draft oven held at 100 °C for one hour.[107] Moisture content was determined as the difference between the original liquid sample weight and the dried sample weight. After each sample was dried, cooled, weighed, re-heated, and weighed again to ensure that all moisture had evaporated, samples were then placed in a muffle furnace and reheated to 525°C for 12-24 hours before weighing again. Ash content was calculated as the difference between the dried weight and furnaced weight.[108]

Calcium Analysis

Calcium was determined using flame atomic absorption spectrometry.[101] The flame absorption spectrometry technique is completed through a specialized instrument. First, a nebulizer creates negative pressure to draw up and transport the liquid sample containing calcium and the solvent through a capillary tube and into the nebulizer. The nebulizer then turns the sample into a very fine mist that is sent into a flame. The flame vaporizes the sample to remove the solvent in a process known as “desolvation”. Free ions and atoms from the calcium are then exposed to a beam of light of a specific wavelength generated by a hollow cathode lamp. The light excites the electrons of the calcium ion, and as a result, the calcium ions absorb light. The decreased light intensity measured by the detector is reported as a measure of absorbance which is then converted into a calcium concentration using a standard curve.[109] All methods of the nutrient analyses are displayed in Table 3.

Table 3: Methods of Nutrient Analyses

Nutrient†	Method of Analysis
Crude fat	Automated acid hydrolysis and solvent extraction
Crude protein	Kjeldahl method and/or combustion
Total carbohydrate	Total carbohydrate (g) = 100 g milk – [total protein (g) + total fat (g) + ash (g) + moisture (g)
Total energy	Total energy (kcal) = (carbohydrate (g) x 4 kcal/g) + (fat (g) x 9 kcal/g) + (protein (g) x 4kcal/g)
Lactose*	Refractive index detector following high pressure liquid chromatography
Moisture	Gravimetric: liquid weight – forced draft oven weight
Ash	Gravimetric: dried weight – furnace weight
Calcium*	Flame atomic absorption spectrometry

†Nutrients reported in g/mg/kcal per 100g liquid/dried product
*Lactose and calcium analyses conducted only in milk

Water Buffalo Milk and Acid Whey Comparisons

Due to only having the triplicate and single samples of Lao PDR water buffalo milk and acid whey, statistical analyses were restricted to basic descriptive statistics. All water buffalo milk and acid whey nutrients were expressed in units per 100 grams of liquid or powder to allow comparisons to other types of mammalian milk.

Water Buffalo Milk Comparisons

Raw, unpasteurized Lao PDR water buffalo milk duplicate results were averaged by nutrient to produce mean values for total energy (kcal), crude fat (g), crude protein (g), total carbohydrate (g), ash (g), moisture (g), lactose (g), and calcium (mg) per 100 grams. Mean values of water buffalo milk nutrients were compared to reference data of raw, unfiltered cow's milk from Egypt.[19] In addition to the mean values, a difference in the nutrient concentrations (reported as a percentage) was calculated as the difference between each Lao PDR water buffalo milk nutrient and the corresponding cow's milk nutrient and dividing this difference by the cow's milk nutrient value and multiplying by 100 [difference (%) = [(water buffalo nutrient-cow's milk nutrient)/cow's milk nutrient)*100]. A negative percentage was interpreted as a lower nutrient concentration in Lao PDR water buffalo milk than cow's milk, whereas a positive percentage represented a higher nutrient concentration in Lao PDR water buffalo milk than cow's milk. Lao PDR water buffalo milk nutrient concentrations were also compared to raw, unpasteurized water buffalo milk nutrient concentrations reported in the literature.[19,79,99,100]

Water Buffalo Milk Liquid Acid Whey Comparisons

Lao PDR water buffalo milk liquid acid whey duplicate results were averaged by nutrient and acid whey type to produce mean values for total energy (kcal), crude fat (g), crude protein (g), total carbohydrate (g), ash (g), and moisture (g) per 100 grams. Individual nutrient concentrations from liquid acid feta and ricotta whey were also combined to calculate one, equal parts-mixed, liquid acid whey with its own set of mean nutrient values. In this setting, the combined samples were not analyzed for energy and nutrient concentrations independently. Additionally, since it was not established how much water buffalo liquid acid whey is derived from each type of water buffalo cheese produced, feta and ricotta whey were combined in equal parts. Because all mozzarella acid whey produced by the Laos Buffalo Dairy is used to make ricotta cheese, feta and ricotta whey are the only two water buffalo milk acid whey types that would potentially be combined to make a protein supplement; hence, only data from these two types of whey were combined.

Lao PDR water buffalo milk liquid acid whey nutrient mean values were compared to reference data of cow's liquid acid whey reported in the USDA FoodData Central.[20] A percent difference was calculated to compare each Lao PDR water buffalo acid whey nutrient concentration to the corresponding cow's acid whey nutrient concentration.

Mean protein concentrations per 100 grams of each liquid acid whey and the combined feta and ricotta acid whey were compared to the United States Dairy Export Council's protein standards for 100 grams of liquid sweet and acid cow's whey. Because sweet whey is often used as a dietary protein modulator and not acid whey, all Lao PDR water buffalo acid whey samples were compared to a "protein standard range" for both acid and sweet dairy cow's whey. The combined and individual liquid acid whey protein averages are reported as grams of protein per 100 grams of liquid water buffalo milk acid whey. Category of protein content for each Lao PDR acid whey sample was determined based on established definitions:[22]

High Protein Content: ≥ 0.85 grams protein/100 g liquid whey

Medium Protein Content: 0.76-0.84 grams protein/100 g liquid whey

Low Protein Content: < 0.76 grams protein/100 g liquid whey

A score of medium to high is comparable to cow's liquid acid and sweet whey and was considered "adequate" to make a powdered whey protein formula.[22]

Water Buffalo Milk Dried Acid Whey Comparisons

Lao PDR water buffalo milk dried acid whey duplicate results were compared to reference data of cow's dried acid whey reported in the USDA FoodData Central.[21]

Mean protein concentrations per 100 grams of each feta, mozzarella, and ricotta dried acid whey and the combined feta-ricotta dried acid whey were compared to the United States Dairy Export Council's protein standards for 100 grams of powdered acid and sweet cow's whey. The combined and individual dried acid whey protein averages were reported as grams of protein per 100 grams of dried Lao PDR water buffalo acid whey. Category of protein content was determined based on established definitions:[22]

High Protein Content: ≥ 11 grams protein/100 g powdered whey

Medium Protein Content: 7-11 grams protein/100 g powdered whey

Low Protein Content: < 7 grams protein/100 g powdered whey

A score of medium to high is comparable to cow's dehydrated acid and sweet whey and was considered an adequate protein source to make a powdered protein supplement.[22]

Chapter 4

Results

Water Buffalo Milk

The concentrations of total energy, crude fat, crude protein, total carbohydrate, lactose, ash, moisture, and calcium in 100 grams of raw, unpasteurized Egyptian dairy cow and Lao PDR water buffalo milk from this study and other published studies are presented in Table 4.[19,79,99,100] Compared to Egyptian dairy cow milk,[19] Lao PDR water buffalo milk contained higher concentrations of total energy, crude fat, crude protein, lactose, and calcium. In contrast, the concentrations of total carbohydrate, ash, and moisture in Lao PDR water buffalo milk were within 10% of those in cow's milk.[19] The nutrient concentrations in Lao PDR water buffalo milk were comparable to those in water buffalo milk reported by Soliman[19], Wong[99], Han et al.[79], and Khan et al.[100], with the exception that the concentrations of lactose and calcium in Lao PDR water buffalo milk were slightly higher.

Table 4: Concentrations of Total Energy, Crude Fat, Crude Protein, Total Carbohydrate, Lactose, Ash, Moisture, and Calcium in 100 grams of Raw, Unpasteurized Dairy Cow and Water Buffalo Milk from This and Other Published Studies

Type of Milk	Total Energy (kcal)	Crude Fat (g)	Crude Protein (g)	Total Carbohydrate (g)	Lactose (g)	Ash (g)	Moisture (%)	Calcium (mg)
Cow's Milk (reference)[19]	71.1	4.14	3.48	4.98	4.70	0.71	86.7	120
Water Buffalo Milk								
Lao PDR Water Buffalo	107	7.64	4.48	5.20	6.39	0.77	85.7	192
Soliman[19]	105	7.52	4.02	5.33	5.02	0.80	82.3	163
Wong[99]	101	7.40	3.80	4.83	4.80	0.80	83.2	-
Han et al.[79]	-	7.16	5.02	-	4.59	0.92	-	179
Khan et al.[100]	-	7.85	3.88	-	4.78	0.71	83.0	-
Difference (%) between Lao PDR Water Buffalo and Cow's Milk*	50.5	84.5	28.7	4.4	36.0	8.45	-1.2	60.1

*Differences between Lao PDR water buffalo and dairy cow milk nutrient concentrations, presented as a percentage, were calculated as [(water buffalo nutrient-cow milk nutrient)/cow milk nutrient] x 100

Water Buffalo Milk Liquid Acid Whey

The concentrations of total energy, crude fat, crude protein, total carbohydrate, ash, and moisture in 100 grams of liquid acid whey from U.S. dairy cow[20] and Lao PDR water buffalo milk and the differences between the two are presented in Table 5. Water buffalo milk liquid acid whey derived from the feta, mozzarella, and ricotta cheese making process contained higher concentrations of total energy and crude fat than cow's milk liquid acid whey.[20] Water buffalo milk feta and mozzarella liquid acid whey contained higher concentrations of crude protein, while water buffalo milk liquid acid whey derived from making ricotta cheese contained 47.4% less protein than cow's milk liquid acid whey.[20] Of the three water buffalo liquid acid whey samples, the feta liquid acid whey contained the highest concentration of total energy (31.3g/100g), crude fat (0.83g/100g), and crude protein (0.99g/100g). The mozzarella liquid acid whey contained the highest concentration of ash (0.67g/100g). The ricotta liquid acid whey contained the lowest concentration of total energy (27.0g/100g), crude fat (0.32g/100g), and crude protein (0.40g/100g). The concentrations of total carbohydrate and moisture were comparable between the three water buffalo liquid acid whey types. Of note, the equal-parts mixture of feta and ricotta liquid acid whey was estimated to contain 29 kilocalories of energy, 0.58 grams of crude fat, 0.70 grams of crude protein, 5.13 grams of total carbohydrate, 0.47 grams of ash, and a moisture content of 93% per 100 grams of liquid whey.

Table 5: Concentrations of Total Energy, Crude Fat, Crude Protein, Total Carbohydrate, Ash, and Moisture per 100 grams of Dairy Cow and Lao PDR Water Buffalo Liquid Acid Whey

Whey Type	Total Energy (kcal)	Crude Fat (g)	Crude Protein (g)	Total Carbohydrate (g)	Ash (g)	Moisture (%)
Cow's Milk Liquid Acid Whey[20] (Reference)	24.0	0.09	0.76	5.12	0.61	93.4
Lao PDR Water Buffalo Milk Liquid Acid Whey						
Feta	31.3	0.83	0.99	4.97	0.43	92.8
Difference (%)*	30.5	822	30.3	-2.39	-29.5	-0.69
Mozzarella	30.4	0.54	0.95	5.45	0.67	92.4
Difference (%)*	26.8	500	25.0	6.45	9.84	-1.10
Ricotta	27.0	0.32	0.40	5.65	0.50	93.1
Difference (%)*	12.6	256	-47.4	10.4	-18.0	-0.30

*Difference between Lao PDR water buffalo and dairy cow's liquid acid whey energy and nutrient concentrations, presented as a percentage, were calculated as [(water buffalo milk whey nutrient-cow's milk whey nutrient)/cow's milk whey nutrient) x 100

Water Buffalo Milk Dried Acid Whey

The concentrations of total energy, crude fat, crude protein, total carbohydrate, ash, and moisture in 100 grams of dried acid whey from U.S. dairy cow[21] and Lao PDR water buffalo milk and the differences between the two are displayed in Table 6. Water buffalo dried acid whey contained higher concentrations of total energy, crude fat, and moisture, but a lower concentration of ash, than cow's milk dried acid whey.[21] Water buffalo feta and mozzarella dried acid whey contained comparable concentrations of crude protein to cow's milk dried acid whey,[21] while the concentration of crude protein in water buffalo ricotta dried acid whey was 55% lower than the concentration of crude protein in cow's milk dried acid whey.[21] Of the three water buffalo dried acid whey samples, the feta dried acid whey contained the highest concentrations of total energy (392 kcal/100g), crude fat (8.75g/100g), and crude protein (11.8g/100g). Water buffalo mozzarella dried acid whey had a lower concentration of total energy (366 kcal/100g) but higher concentrations of ash (7.89g/100g) and moisture (7.89g/100g) than the feta and ricotta dried acid whey samples. In contrast, water buffalo ricotta dried acid whey contained lower concentrations of crude fat (4.85g/100g), crude protein (5.28g/100g), and moisture (6.65g/100g) than the feta and mozzarella dried acid whey samples. The equal-parts mixture of feta and ricotta dried acid whey was estimated to contain 381 kilocalories of energy, 6.8 grams of crude fat, 8.54 grams of crude protein, 71.5 grams of total carbohydrate, 6.08 grams of ash, and a moisture content of 7.13% per 100 grams of dried whey.

Table 6: Concentrations of Total Energy, Crude Fat, Crude Protein, Total Carbohydrate, Ash, and Moisture in 100 grams of Dairy Cow and Lao PDR Water Buffalo Dried Acid Whey

Whey Type	Total Energy (kcal)	Crude Fat (g)	Crude Protein (g)	Total Carbohydrate (g)	Ash (g)	Moisture (%)
Cow's Milk Dried Acid Whey[21] (Reference)	339	0.54	11.7	73.5	10.8	3.5
Lao PDR Water Buffalo Milk Dried Acid Whey						
Feta	392	8.75	11.8	66.5	5.31	7.61
Difference (%)*	15.7	1520	0.68	-9.43	-50.7	117
Mozzarella	366	5.74	11.2	67.3	7.89	7.89
Difference (%)*	7.85	963	-4.31	-8.43	-26.7	125
Ricotta	370	4.85	5.28	76.4	6.85	6.65
Difference (%)*	9.22	797	-55.0	4.0	-36.4	89.5

*Difference between Lao PDR water buffalo and dairy cow's dried acid whey energy and nutrient concentrations, presented as a percentage, were calculated as [(water buffalo milk whey nutrient-cow's milk whey nutrient)/cow's milk whey nutrient) x 100

Water Buffalo Milk Liquid and Dried Acid Whey Protein Categories

The protein categories of Lao PDR water buffalo milk liquid and dried acid whey, based on the protein standards per 100 grams of cow's milk liquid and dried sweet and acid whey,[22] are presented in Table 7. Lao PDR water buffalo milk feta and mozzarella liquid acid whey exceeded the 0.85 grams of protein per 100 grams of liquid whey required to be classified as a "high" source of protein. In contrast, ricotta liquid acid whey and the equal-parts mixture of feta and ricotta liquid acid whey contained less than 0.76 grams of protein per 100 grams of liquid whey and were classified as a "low" source of protein.

Lao PDR water buffalo milk feta and mozzarella dried acid whey samples exceeded the 11 grams of protein per 100 grams of dried whey required to be classified as a "high" source of protein. Lao PDR water buffalo milk ricotta dried acid whey contained less than 7 grams of protein per 100 grams of powder and was classified as a "low" source of protein. The equal-parts mixture of feta and ricotta dried acid whey contained 8.54 grams of protein per 100 grams of powder and was classified as a "medium" source of protein.

Table 7: Protein Category of Lao PDR Water Buffalo Liquid and Dried Acid Whey

Whey Type	Liquid Acid Whey Crude Protein (g/100g)	Dried Acid Whey Crude Protein (g/100g)	Protein Category (Liquid Whey*/Dried Whey†)
Feta	0.99	11.8	High/High
Mozzarella	0.95	11.2	High/High
Ricotta	0.40	5.28	Low/Low
Feta-Ricotta Mixture**	0.70	8.54	Low/Medium

*Criteria for Protein Category: Liquid Whey[21]
High Protein Content: ≥ 0.85 grams protein/100 g liquid whey
Medium Protein Content: 0.76-0.84 grams protein/100 g liquid whey
Low Protein Content: < 0.76 grams protein/100 g liquid whey

†Criteria for Protein Category: Freeze-Dried Whey[21]
High Protein Content: ≥ 11g protein/100 g dried whey
Medium Protein Content: 7-11g protein/100 g dried whey
Low Protein Content: < 7g protein/100 g dried whey

**All mozzarella liquid acid whey produced by the Laos Buffalo Dairy is used to make ricotta cheese; therefore, only feta and ricotta acid whey would potentially be combined to make a protein supplement.

Chapter 5

Discussion

Determining the nutritional composition of Lao PDR water buffalo milk and its liquid and reconstituted powdered acid whey byproducts are the first steps to deciding whether a powdered protein supplement, that is safe for human consumption, should be developed. Lao PDR water buffalo milk contained higher concentrations of total energy, crude fat, crude protein, lactose, and calcium, than cow's milk.[19] In addition, the nutrient concentrations reported in Lao PDR water buffalo milk agree with the concentration of nutrients in water buffalo milk reported by Soliman[19], Wong[99], Han et al.[79], and Khan et al.[100]; though, the lactose and calcium concentrations of the Lao PDR samples are slightly higher.

Throughout the literature, the higher concentrations of nutrients in water buffalo milk are attributed, in part, to the milk's lower moisture content[19,79,99,100]; however, the moisture content of Lao PDR water buffalo milk was similar to that of cow's milk (85.7% vs 86.7%, respectively).[19] In addition, Lao PDR water buffalo milk had a comparable moisture content to water buffalo milk previously reported in the literature (85.7% Lao PDR water buffalo milk vs 82.3%[19], 83.2%[87] and 83%[100], a difference of 3%-4%). Further research with a larger number of Lao PDR water buffalo milk samples collected across annual seasons and additional nutrient analyses are needed to confirm our results and determine if the concentration of nutrients in Lao PDR water buffalo milk are related to the concentration of moisture or other properties within the milk.

The higher concentration of total energy in Lao PDR water buffalo milk (107 kcal/100g) than cow's milk (71.1 kcal/100g) is likely due to the higher concentration of crude fat (7.64 g/100g vs 4.14g/100g, respectively). Fat provides 9 kilocalories of energy per gram, whereas carbohydrate and protein provide 4 kilocalories of energy per gram. Compared to cow's milk,[19] Lao PDR water buffalo milk contained a similar amount of total carbohydrate (5.20g/100g water buffalo milk vs 4.98g/100g cow's milk) and 28.7% more protein (4.48/100g water buffalo milk vs 3.48/100g cow's milk). Because Lao PDR water buffalo milk contained 85% more crude fat than cow's milk,[19] the higher concentration of total energy is logical.

The concentration of lactose, the primary carbohydrate in mammalian milk, was slightly higher in Lao PDR water buffalo milk than cow's milk.[19] In addition, the concentration of lactose from this study differed from the concentration of lactose reported in the literature by 1.8 grams, or a difference of 39%. Typically, lactose comprises 91-94% of the total carbohydrate in cow's milk.[19,110] Due to lactose being the main type of carbohydrate found in mammalian milk, we expected the concentration of lactose to be equal to or slightly less than the concentration of total carbohydrate in our water buffalo milk samples. However, this was not the case. Instead, we observed a higher concentration of lactose (6.39g/100g) than total carbohydrate (5.20g/100g) in our Lao PDR water buffalo milk samples. This unexpected observation was likely the result of combined measurement errors. In our study, lactose was measured directly and independent of the other water buffalo milk nutrients. In contrast, the concentration of total carbohydrate was estimated as the difference in weight of 100 grams of milk and the measured weight of each independently measured milk constituent, such that total carbohydrate (g) = 100 g milk – [total protein (g) + total fat (g) + ash (g) + moisture (g)].[101] When compared, the difference in the concentration of lactose and total carbohydrate in Lao PDR water buffalo was 1.19 grams in 100 grams of milk. Because there is inherited measurement error in each of these analyses, the sum of these errors could account for the difference in concentrations of lactose and total carbohydrate. Further research using a larger number of Lao PDR water buffalo milk samples collected throughout the year is needed to confirm the concentrations of nutrients, including lactose, in Lao PDR water buffalo milk.

Due to the concentration of total carbohydrate from this study being comparable to that reported in the literature (5.20g/100g vs 5.33g[19] and 4.83g[99]/100 g, respectively), the concentration of lactose in Lao PDR water buffalo milk may be comparable as well. Overall, further research using a larger number of Lao PDR water buffalo milk samples collected throughout the year is needed to confirm the concentrations of nutrients, including lactose, in Lao PDR water buffalo milk.

Because the concentration of total carbohydrate was comparable between Lao PDR water buffalo milk and cow's milk,[19] it is possible that the concentration of lactose is comparable between the two mammal milks as well. Confirming the concentration of lactose in Lao PDR water buffalo milk will be interesting, as it is stated that water buffalo milk contains a lower concentration of lactose than cow's milk,[75] and therefore, maybe easier to digest. A connection between the digestibly of water buffalo milk and its concentration of lactose has not been established in previous research. However, if water buffalo milk and cow's milk contain similar concentrations of lactose, more research will be needed to determine why water buffalo milk is perceived to be tolerated better by individuals who do not tolerate cow's milk.

Based on our nutrient analyses, Lao PDR water buffalo milk contained 60% more calcium than cow's milk[19] (192mg/100g vs 120mg/100g, respectively). Further, the amount of calcium in 8 ounces of Lao PDR water buffalo milk provides 36%-67% of the daily calcium requirements, established by the Institute of Medicine, for children 1-18 years of age.[111] In comparison, 8 ounces of cow's milk provides 23%-42% of the daily calcium requirements for children in the same age range.[111] Since Lao PDR water buffalo milk provides more than 20% of the estimated daily calcium requirement, it is regarded as an "excellent" source of dietary calcium for growing children.[112] Though Lao PDR water buffalo milk and water buffalo milk described in previously published studies contain higher concentrations of calcium than cow's milk,[19] there is no convincing explanation of this finding. The only explanation provided in the literature is the lower moisture content of water buffalo milk.

The concentration of calcium in mammalian milk can be affected by the age of the lactating mammal, the number of previous lactation cycles, and the day during the lactation period when milk samples are derived.[113,114] Because the Lao PDR water buffalo milk samples were collected as pooled samples from many animals, it is difficult to explain the higher concentration of calcium compared to that reported in the literature. The amount of calcium in a dairy cow's diet has been found to minimally affect the concentration of calcium in cow's milk[115], though this same relationship may not apply to water buffalo. In Luang Prabang, where the Laos Buffalo Dairy is located, the concentration of calcium in unfiltered water is naturally high.[75,116] Since water buffalo located at the Laos Buffalo Dairy drink unfiltered water, the amount of calcium consumed by these buffalo may be higher than the amount of calcium consumed by water buffalo raised in other areas. It could be that the presumably higher dietary calcium consumed by Lao PDR water buffalo does indeed influence the concentration of calcium in their milk; though more research is needed to confirm this hypothesis.

Nutrient concentrations of Lao PDR water buffalo milk liquid acid whey varied depending on the type of acid whey analyzed. Compared to cow's milk liquid acid whey,[20] water buffalo milk liquid acid whey derived from feta, mozzarella, and ricotta cheese contained higher concentrations of total energy and crude fat. This could be because liquid acid whey, when unfiltered, contains lipids from whole milk after its separation from casein.[22] Since Lao PDR water buffalo milk contained a higher concentration of crude fat than cow's milk, Lao PDR water buffalo milk liquid acid whey would also contain a higher concentration of crude fat than cow's milk liquid acid whey. Additionally, the cow's milk liquid acid whey may have been filtered to remove or decrease the amount of fat in the analyzed product.

Compared to cow's milk liquid acid whey,[20] Lao PDR water buffalo milk liquid acid whey derived from feta and mozzarella cheese contained higher concentrations of crude protein. In contrast, Lao PDR water buffalo milk liquid acid whey derived from ricotta cheese and the mixture of equal-parts feta and ricotta acid whey contained a lower concentration of crude protein than cow's milk liquid acid whey.[20] The difference in protein concentration, along with the slight variations in total carbohydrate and ash concentrations, may be attributed to the unique cheese-making process that each whey sample was derived from. For example, water buffalo ricotta cheese is produced using the residual liquid whey derived from making mozzarella cheese. Because ricotta acid whey is not formed directly from the separation of fresh milk into casein and whey, ricotta acid whey contains lower concentrations of crude fat and crude protein than the other liquid acid whey products. As there is no published data on the concentration of protein in different types of cow's milk liquid acid whey or water buffalo milk liquid acid whey, more research is needed to confirm our results.

The concentration of total carbohydrate in the three water buffalo milk liquid acid whey samples differed, at most, by 0.53 grams compared to cow's milk liquid acid whey.[20] Based on these results, there is a minimal difference in the concentration of total carbohydrate, and possibly the concentration of lactose, between the two mammalian acid whey products. Further analyses are needed to determine the concentration of lactose in each type of water buffalo liquid acid whey. Due to water buffalo ricotta liquid acid whey containing the highest concentration of total carbohydrate, it is possible that ricotta acid whey also contains the highest concentration of lactose, compared to the other types of water buffalo acid whey.

The concentrations of ash in water buffalo milk liquid acid whey derived from feta, mozzarella, and ricotta cheese were comparable to the concentration of ash reported in cow's milk liquid acid whey.[20] However, feta (0.43g/100g) and ricotta (0.50g/100g) liquid acid whey, because of their ash concentrations, may have desirable drying characteristics. When found in high concentrations, ash, or the mineral content of mammalian milk, prevents whey from drying correctly, regardless of the drying procedure, and leads to early rancidity of powdered whey protein.[17,52] Because cow's milk sweet whey contains a lower concentration of ash than cow's milk acid whey, it is often dried and used as a dietary protein modulator.[22] Interestingly, water buffalo feta and ricotta liquid acid whey contained lower concentrations of ash than cow's milk liquid sweet whey (0.53g/100g)[50] and, therefore, may have favorable drying properties similar to cow's milk sweet whey.

Lao PDR water buffalo milk dried acid whey derived from feta, mozzarella, and ricotta cheese contained higher concentrations of total energy (392, 366 and 370 kcal/100g, respectively) and crude fat (8.75, 5.74 and 4.85g/100g, respectively) than cow's milk dried acid whey (339 kcal; 0.54g crude fat/100g).[21] In addition, each type of Lao PDR water buffalo dried acid whey contained a higher moisture content and a lower concentration of ash than cow's milk dried acid whey.[21]

Similar to Lao PDR water buffalo milk, the higher concentration of total energy in Lao PDR water buffalo milk dried acid whey is likely due to the higher concentration of crude fat in these samples. Although a higher concentration of total energy is favorable if the whey supplement is to be used to rehabilitate malnourished individuals, crude fat in dried whey powders is susceptible to degradation, and when exposed to oxygen and moisture, which can result in the rancidity of the whey protein supplement.[22,91] Because of this, further research is needed to determine the best packaging strategies to prolong the shelf life of Lao PDR water buffalo milk dried acid whey when high concentrations of crude fat remain after dehydrating. Typically, cow's milk whey is filtered before dehydrating to reduce the total concertation of fat to 1-2 grams per 100 grams of powder, which allows storage in non-specialized airtight packaging with a shelf life up to 9 months.[91]

Lao PDR water buffalo milk dried acid whey contained 89%-125% more moisture than cow's milk dried acid whey.[21] The higher percentage of moisture in Lao PDR water buffalo milk dried acid whey may be due to differences in drying procedures. The freeze-drying method is not commonly used to dehydrate liquid dairy products, and instead, a spray drier or drum roller is preferred. Furthermore, the USDA's FoodData Central did not specify which drying method was used to obtain the reference data for cow's milk dried acid whey. Thus, a drying method other than freeze-drying could have been used. In addition, the goal moisture content for a dried dairy product is less than 5%.[93] The concentration of moisture in the water buffalo dried acid whey samples (6.65g-7.89g/100g) suggests that the acid whey was not fully dehydrated, and therefore, Lao PDR water buffalo milk liquid acid whey may require a longer drying time than cow's milk liquid acid whey.

Lao PDR water buffalo milk dried acid whey, with the exception of whey derived from feta cheese, contained a lower concentration of crude protein than cow's milk dried acid whey (mozzarella 11.2g and ricotta 5.28g/100g vs 11.7g/100g cow's dried whey).[21] The concentrations of crude protein, and other nutrients, may be skewed due to the higher concentration of moisture in the water buffalo acid whey. If the Lao PDR water buffalo acid whey samples had been dehydrated to a comparable moisture content as the cow's dried acid whey, all nutrient concentrations, specifically crude protein, would likely have been even higher per 100 grams of finished whey powder.

To estimate the concentration of nutrients that would have been in our Lao PDR water buffalo dried acid whey samples if they had been dehydrated to a comparable moisture content as the cow's dried acid whey, the gram weight of each fully dehydrated water buffalo acid whey was determined. The gram amount of each water buffalo dried acid whey was then "rehydrated" with 3.5 grams of water, as this is the content of moisture in the cow's dried acid whey. As a result, "adjusted" water buffalo dried acid whey gram amounts of powder were calculated as = (100g dried whey - grams of water remaining in water buffalo dried acid whey) + 3.5g water. The adjusted weight in grams of powder was then used to estimate the adjusted concentrations of nutrients in each water buffalo acid whey when dehydrated to a comparable moisture content of the cow's dried acid whey. This was calculated as adjusted nutrient concentration = [(old nutrient concentration x grams of dried water buffalo whey when rehydrated with 3.5g water)/grams of water buffalo dried acid whey when all moisture is removed].

Using this calculation, water buffalo feta dried acid whey would contain 407 calories, 9.1 grams of crude fat, 12.2 grams of crude protein, 69 grams of total carbohydrate, and 5.5 grams of ash. Water buffalo mozzarella dried acid whey would contain 378 calories, 6.0 grams of crude fat, 11.6 grams of crude protein, 69.9 grams of total carbohydrate, and 8.2 grams of ash. And, water buffalo ricotta dried acid whey would contain 384 calories, 5.0 grams of crude fat, 5.5 grams of crude protein, 79.3 grams of total carbohydrate, and 7.1 grams of ash.

According to the protein standards for 100 grams of liquid acid and sweet cow's whey, as defined by the United States Dairy Export Council,[22] only Lao PDR water buffalo feta (0.99g protein/100g liquid) and mozzarella (0.95g protein/100g liquid) liquid acid whey contained "adequate" concentrations of protein (defined as 0.75g protein/100g liquid). These results suggest that feta and mozzarella water buffalo liquid acid whey would be appropriate to dehydrate and powder into a shelf-stable source of protein. However, it is important to note that at the Laos Buffalo Dairy, all mozzarella liquid acid whey is used to produce ricotta cheese.[75] Because of this, Lao PDR water buffalo feta liquid acid whey would be the best option to yield a high protein powdered whey product.

Surprisingly, the protein classifications of Lao PDR water buffalo dried acid whey differed from the protein classifications of Lao PDR water buffalo liquid acid whey. Water buffalo feta (11.8g protein/100g powder) and mozzarella (11.2g protein/100g powder) dried acid whey were classified as "adequate" sources of protein to form a shelf-stable whey product. However, the mixture of feta and ricotta dried acid whey also approached an "adequate" concentration of protein (0.70g protein/100g powder). Accordingly, whey powders derived from feta, mozzarella, or an equal-parts mixture of feta and ricotta acid whey would be appropriate to form a shelf-stable source of protein. Because Lao PDR water buffalo produce a limited amount of milk, and further, a substantial amount of cheese must be made to derive enough liquid acid whey to produce a powdered supplement, using a mixture of equal-parts feta and ricotta whey will be the most efficient option to capture all of the available protein for use to create a powdered protein supplement.

Although Lao PDR water buffalo liquid acid whey derived from the cheese-making process will be appropriate to dehydrate, it is important to note the potential costs and concerns of developing a powdered whey product. To create a stable, easily solulizable dried product, whey must be filtered to decrease the concentration of lactose, fat, and other water-soluble minerals.[22,90,91] This requires a whey processing system that can perform ultra- and diafiltration. A small ultrafiltration system can cost between $20,000-$25,000 United States dollars, and this estimate does not include the cost of additional equipment, such as whey permeate holding containers; yearly repairs; the mechanical energy demands; or the filter replacements.[117] In addition, drying equipment must be purchased. The expense of a small spray dryer that can dehydrate 5-8 liters of whey per hour is $67,500 United States dollars.[118] In contrast, a small freeze-dryer that can dehydrate 15 liters of fluid per 20-40 hours is $7,500 United States dollars.[119] Creating an acceptable dietary protein supplement derived from Lao PDR water buffalo milk will likely be difficult, as delivering, installing, and preforming continual maintenance on the filtration and drying equipment may be challenging in this low-resource country. Unfortunately, powdering unfiltered Lao PDR water buffalo liquid acid whey using simple drying equipment, such as a home dehydrator or oven, will not be appropriate. Specifically, home dehydrators will cause heat damage to the whey protein and will result in large whey particles that taste chalky and have a low solubility.[90] Because the volume of water buffalo whey produced daily by the Laos

Buffalo Dairy has not been established, further research is needed to determine if the production of a powdered whey supplement in Lao PDR will be an affordable source of supplemental energy, protein, and other nutrients.

Another potential concern is the cultural acceptability of the dietary energy, protein, and nutrient modulator. Individuals living in Lao PDR may be wary of consuming water buffalo milk and its acid whey byproduct, and therefore, these products may be difficult to incorporate into the diets of malnourished individuals. Throughout Lao PDR, some families believe that they will assume the personality traits of a water buffalo when the animal’s milk is consumed. It is also believed that consuming water buffalo milk will make the “spirits” upset, as mammalian milk is intended only for the mammal’s offspring.[120] Although these same beliefs may not apply to water buffalo acid whey, further public education will be needed to promote the benefits of incorporating water buffalo milk and its acid whey byproduct into the diets of malnourished children and adults.

Chapter 6

Conclusion

Determining the nutritional composition of Lao PDR water buffalo milk and its liquid and reconstituted powdered acid whey byproducts, generated as part of the cheese-making process, are the first steps to determine if developing a locally sourced, shelf-stable, powdered protein supplement that is safe for human consumption is reasonable. To answer this question, we undertook the following aims and tested the following hypotheses:

Aim 1: Determine the energy, macronutrient, moisture, ash, and calcium concentrations of raw, unpasteurized Lao PDR water buffalo milk and compare these values to available reference data derived from raw, unpasteurized cow's milk in Egypt.[19]

Hypothesis 1: Raw, unpasteurized Lao PDR water buffalo milk will have higher concentrations of energy, protein, fat, carbohydrate, lactose, calcium, and ash and a lower moisture content than what has been reported for raw, unpasteurized dairy cow's milk in Egypt.[19]

Conclusion: Lao PDR water buffalo milk contained higher concentrations of total energy, crude protein, crude fat, lactose, and calcium than dairy cow's milk and had a similar concentration of total carbohydrate, ash, and moisture.[19] Because of this, we confirm, in part, this first hypotheses.

Aim 2: Determine the energy, macronutrient, moisture, and ash composition of the liquid acid whey fraction of pasteurized Lao PDR water buffalo milk produced as a part of the soft cheese-making process and compare these values to reference data of liquid acid whey derived from cow's milk as reported in the United States Department of Agriculture (USDA) FoodData Central nutrient database.[20]

Hypothesis 2 (a): Pasteurized Lao PDR water buffalo milk liquid acid whey will have higher concentrations of energy, fat, carbohydrate, and ash and a lower moisture content than what has been reported for dairy cow's milk liquid acid whey as reported in the USDA FoodData Central nutrient database.[21]

Hypothesis 2 (b): The protein concentration of pasteurized Lao PDR water buffalo milk liquid acid whey will be at least 0.76 grams of protein per 100 grams of liquid acid whey and will meet the protein concentration standard of liquid acid whey derived from dairy cow's milk as reported by the United States Dairy Export Council.[22]

Conclusion: Lao PDR water buffalo milk liquid acid whey contained higher concentrations of total energy and crude fat than dairy cow's milk liquid acid whey.[20] Further, the concentration of total carbohydrate, ash, and moisture in Lao PDR water buffalo milk liquid acid whey was comparable to the concentrations in cow's milk liquid acid whey.[20] In addition, only water buffalo feta and mozzarella liquid acid whey contained at least 0.76 grams of protein per 100 grams of liquid acid whey to meet the protein concentration standard of liquid acid whey derived from dairy cow's milk.[22] Therefore, we confirm, in part, our second hypotheses.

Aim 3: Determine the energy, macronutrient, moisture, and ash composition of the reconstituted dried acid whey fraction of pasteurized Lao PDR water buffalo milk produced as a part of the soft cheese-making process and compare these values to reference data of dried acid whey derived from cow's milk in the USDA FoodData Central nutrient database.[21]

Hypothesis 3 (a): Pasteurized Lao PDR water buffalo milk reconstituted dried acid whey will have higher concentrations of energy, fat, carbohydrate, and ash than dairy cow's milk reconstituted dried acid whey as reported in the USDA FoodData Central nutrient database.[21]

Hypothesis 3 (b): The protein concentration of pasteurized Lao PDR water buffalo milk reconstituted dried acid whey will be at least 7 grams of protein per 100 grams of powdered acid whey or 7% by weight volume, thereby aligning with the protein concentration of powdered acid whey derived from dairy cow's milk as reported by the United States Dairy Export Council.[22]

Conclusion: Lao PDR water buffalo milk dried acid whey contained higher concentrations of total energy and crude fat. However, it also contained a higher concentration of moisture, a lower concentration of ash, and a similar concentration of total carbohydrate, compared to cow's milk dried acid whey.[21] Furthermore, Lao PDR water buffalo milk ricotta dried acid whey contained less than 7 grams of protein per 100 grams of reconstituted dried acid whey, which was lower than the protein concentration of dried acid whey derived from dairy cow's milk.[22] Only water buffalo dried feta, mozzarella, and the equal-parts mixture of feta and ricotta acid whey contained "adequate" concentrations of protein. Therefore, we confirm, in part, our third hypotheses.

Practice Implications

Based on the results from this study, we suggest that it is possible to produce a powdered source of dietary energy, protein, and other nutrients from Lao PDR water buffalo milk. Individually, feta and mozzarella water buffalo dried acid whey are appropriate for this purpose. However, because mozzarella liquid acid whey is used to produce ricotta cheese, using a mixture of equal-parts water buffalo feta and ricotta acid whey will be the most efficient option to capture all of the available protein in what historically would be a discarded by-product of the cheese manufacturing process. Additionally, since water buffalo milk and its acid whey byproduct contain high amounts of protein and other minerals, these dietary modulators are not appropriate to provide as an exclusive source of nutrition to infants under the age of one year and if provided, should only be used with medical supervision.[60] Instead, at least initially, this dietary modulator should only be used as a protein supplement for children and adults who are malnourished and who can tolerate a higher renal solute load. And, as with any nutritional rehabilitation therapy, reintroduction of foods and nutritionally-dense supplements should only be performed with medical supervision using specific re-feeding protocols to minimize the risk of medical complications, such as refeeding syndrome.

Teaching children and adults how to safely consume the water buffalo dried acid whey will be crucial. Because families rely on unfiltered, potentially contaminated water within their homes,[4] education on how to acquire clean, safe water is needed. Of note, foods that are made with hot or previously boiled water or liquids, such as soups or rice porridge, will be the best options to safely rehydrate the powdered whey protein. Overall, for the treatment and/or prevention of malnutrition, children and adults must verbally be taught or given written directions/illustrations on how to properly use and safely rehydrate this powdered source of energy, protein, and other nutrients.

Limitations of the Study

One limitation of this study is the small number of Lao PDR water buffalo milk and acid whey samples analyzed. A larger number of Lao PDR water buffalo milk and acid whey samples, collected across the different seasons, would have allowed us to provide more generalized results that were potentially more reliable and precise.

Another limitation of this study is our reference data. To compare Lao PDR water buffalo milk and acid whey to cow's milk and acid whey, the best practice would have been to analyze samples of Lao PDR cow's milk and acid whey at Mahidol University at the same time and with the same techniques as the Lao PDR water buffalo milk and acid whey to establish our own reference data. This study design would ensure that the same testing and drying procedures were used to compare the two mammalian milks and their acid whey products. Instead, Lao PDR water buffalo products were compared to dairy cow products using previously published data. Raw, unpasteurized Lao PDR water buffalo milk was compared to raw, unpasteurized dairy cow's milk produced and analyzed in Egypt, as this was the only nutrient analysis available on raw cow's milk that included all of the necessary comparison data. Because there is no previously published data on the concentration of nutrients from different types of cow's milk acid whey, each type of Lao PDR water buffalo milk acid whey was compared to reputable, yet generalized, data on cow's milk acid whey derived from the USDA's FoodData Central. In addition, the USDA's FoodData Central did not specify which filtration or drying methods were used to obtain the reference data for cow's milk dried acid whey, and thus, a drying method other than freeze-drying could have been used as well as an ultrafiltration process.

Finally, the long 50-hour period between the time of collecting the milk and acid whey samples from a kitchen refrigerator and traveling to Mahidol University in Bangkok, Thailand may have affected our samples, and therefore, our test results. Although the samples of Lao PDR water buffalo milk and acid whey remained on ice in an insulated cooler while transported, we did not have a thermometer to confirm that the milk and acid whey remained below a temperature of 4°C or less.

Strengths of the Study

Despite these limitations, this study had several strengths. First, we demonstrated that research requiring complex sample collection and laboratory analyses can be conducted in a low-resource country such as Lao PDR. Although the samples of water buffalo milk and acid whey required transportation to a neighboring country to undergo nutrient analyses, the distance traveled was realistic. The analytical techniques and procedures used were also conventional for our purposes. Another strength is that the water buffalo milk and acid whey samples were collected, by professionals, from pooled sources. The pooled water buffalo milk samples analyzed as part of this research came from two different breeds of water buffalo at different lactation stages, representing the typical commercial water buffalo milk available in Lao

PDR. These factors allowed us to collect suitable water buffalo milk and acid whey samples for the nutrient analyses. Finally, this project was unique, in that no other research has been reported on Lao PDR water buffalo milk and whey or its nutrient concentrations. Additionally, the nutrient concentrations of Lao PDR water buffalo milk were analyzed in three different milk phases: raw, unfiltered/non-homogenized milk; the liquid acid whey fraction of milk; and the dried acid whey fraction of milk.

Further Research

Until now, the concentration of nutrients in Lao PDR water buffalo milk and its acid whey fraction have not been established. Though the concentration of nutrients in water buffalo milk derived from other Asian countries has been reported in other publications, further research is needed to confirm the results of our study. Specifically, nutrient analyses on the concentration of lactose and moisture will verify our results that water buffalo milk contains a comparable concentration of these nutrients as dairy cow's milk. Because the concentration of nutrients in the different types of Lao PDR water buffalo acid whey have not previously been established or published, further research to confirm our results is needed.

Finally, additional research is needed to optimize the process to filter, powder, and package whey protein derived from Lao PDR water buffalo. Additionally, further research is needed to determine if incorporation of Lao PDR water buffalo milk and a powdered protein supplement derived from Lao PDR water buffalo whey into the diets of malnourished children and adults improves their nutritional status. Once the process is enhanced and the powdered whey supplement is deemed safe for human consumption, research should be conducted to determine if this dietary supplement improves the nutritional status of those at risk for malnutrition or who are malnourished

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