1 General Introduction

Milk and dairy products have historically contributed to the improvement of human health because of their inter-dependence between diet tradition and consumer choices, research and food industry. In the last decades, the food market and research institutes have faced some denigrating lobbies, like those carried out by the website notmilk.com. A large portion of general literature and some of the scientific too, have raise concerns of milk intake on health issues, in particular for cardiovascular diseases in developed countries. Apart from traditions and the generally recognition of milk as being a component of a good nutrition, the power of media, lobbies along with some wrong medical indication have therefore influenced the consumer choices towards alternatives to dairy products.

Anyway, dairy products can take advantage of considerable portion of the public opinion and by most of the health agency which are well aware of the scientific evidence about good health status that can arise from nutrition of high quality dairy products. This context allows dairy industries to deliver their products in health-promoting system, i.e. *designer* milk as stated by Boland *et al.* (2001).

The quality of milk is linked both to internal factors, the breed of cow, and external, like feeds, seasonal changes, milking frequency and breeding. Among possible techniques capable of changing milk composition, the improvement and the management of animal feed is one of the most used and suited. Each market and region has its peculiarity thanks to typical habits and nutrition deficiency or exceeding, so each fortification program needs to calibrate different type of knowledge, both from animal and human sciences. Moreover, it is to be considerer that each element transfer differently from feed to milk, for instance, iodine and selenium have major transfer efficiency from feed to milk than other microelements, such as iron and manganese (Knowles *et al.,* 2006). Plenty of science evidence point out micronutrients (iron, manganese, copper, zinc, iodine and selenium) acute or sub-deficiency, and the challenge of feed and dairy industries is to find, for a determined region or nation, a good ratio between human nutrition, animal welfare and food fortification feasibility. Feed fortification programs show a good cost-effective ratio because they are concomitantly part of the animal health care. Besides humans, animals have to get advantages from feed-for-food healthy policies. Regarding animal welfare, Bertoni (2009) suggests that is possible to find some area in which compromise the *ethical* and *useful tool* approaches of animals; the chain *feed into milk* should be an area where compromising the *ethical* and *useful tool* approaches between humans and animals welfare.

Feeds have been also involved in various food scandals, like BSE, dioxin, and recently melamine. Illegal actions or bad management of animal feeding are the causes that expose consumers to different, and sometimes unpredictable, health risks. The mammary gland is an excretory organ capable of transferring several molecules and micro-organism from feed to milk. Moreover, each un-desirable nutrient or contaminant in feeds undergoes the digestive process, with the consequence that it could be transferred into milk chemically or physically modified. Before than humans, ethically and economically speaking, we should consider that animal is exposed *in primis* to health hazards.

High quality and designed feeds stand for health and wealth. Bad and adulterated feeds stand for diseases and loss.

Feed and dairy industries need sound analytical supports in order to certify and to verify rapidly their products. In the grass-to-glass engagement (Knowles *et al.,* 2006), in absence of a rapid and efficient official methods of analysis, chemists and food technologists have the rule to implement and to validate new analytical methods capable of supporting the production process and the nutrition demands. Each operation would reduce its efficiency if the quality control slows the industrial process, or if the chemical source of the implemented substance is unknown and/or undetectable.

1.1 A justification for the union of quality and risk: their etymology

According to the Compact Oxford English Dictionary (available at http://www.askoxford.com) the definitions of quality and risk are,

quality: the degree of excellence of something as measured against other similar things / general excellence / a distinctive attribute or characteristic / (archaic) high social standing;

risk: a situation involving exposure to danger / the possibility that something unpleasant will happen / a person or thing causing a risk or regarded in relation to risk.

Despite the definitions of quality and risk are different and they seem antithetic, from their etymology we could find a common point. The origins of these terms are the Latin words *qualitas* and *reseco*, respectively. *Qualitas* was coined by Cicerone for traducing the Greek *poiótes* (Ποιότης) by Plato (Castiglioni and Mariotti, 1966). The notion of quality (or *poiótes*) is very extensive and with difficulty can be reduced to a unique concept; one of its philosophic definitions says that *quality* is the merge of different *properties* of a particular object or condition (Abbagnano, 1971). This doctoral thesis, obviously, do not elaborate on the philosophic discussion about the Aristotelic categories of quality, the definitions by Newton and Wolff, and on the four concepts of the Stoic category "*poia"* (Reesor, 1972), but it is important to underline the meaning of quality as "*qualification"*. Being *qualified* means to have the competence, i.e. the "*dispositional quality*", to perform a specific task or to achieve a given goal. However, sometimes the term *qualified* stands only for "*limited"* or "*characterized by given conditions"*, as used in legal language (Abbagnano, 1971). Through the concept of qualification we could link the risk to the quality as follows. Considering the food chain, the quality should be *limited* to the achievement of human health and animal/environment welfare, due to the *given conditions* of goodness of feed/food management. The previous sentence can be seen as a sum of certain *properties* within a multi-factorial approach to quality. Moreover, if we consider each property, limitation or condition as a positive natural number, and the quality the number given by their sum, the existence of some negative values that move away from the achievement of quality could be possible. What about these negative values? The Latin *reseco* stands for cut away, exclude, suppress, restrain. At this point, the risks could be seen as negative numbers which restrain the objective of the different qualities given by specific properties and limitations.

The above arguments are meant to justify the tasks of this thesis, that, apparently in contrast with each other, are linked by the qualification of feed into milk chain as a tool for human health.

1.2 Thesis structure and aim of the work

The present work is divided into four manuscripts with the aim to study three different aspects of dairy (milk and cheese) and feed industries cited in the paragraph 1. The three tasks can be considered as independent case studies within the quality management of feed-for-food system. Two cases regard the animal feeding: one is about the fortification of feeds with iodine and selenium to improve the quality of milk, whey and cheese; the other one investigates the contamination of feeds with melamine as risk factor for dairy industries and human health. The third case study of this work is a corollary to the iodine-linked quality of dairy products: i.e. a comparison between analytical procedures for the determination of iodine in raw milk, and a speciation study of iodine in fortified raw milk.

First manuscript (Journal article)

Iodine and selenium carry over in milk and cheese in dairy cows: effect of diet supplementation and milk yield.

Published by Cambridge University Press in *Animal* (2010), 4:147-155

The manuscript is divided into two experiments. The first had the aim of studying the effect of different levels of inorganic source of iodine and selenium in the diet on carry over in milk, and their transfer from milk into cheese. The second experiment aim was to evaluate the effect of milk yielding and stage of lactation on iodine and selenium milk concentration and carry over. Discussions were developed about nutrition values of milk and cost-efficiency of selenium sources (sodium selenite and selenized-yeast) in animal feeds.

Second manuscript (Journal article)

Transfer of melamine from feed to milk and from milk to cheese and whey in lactating dairy cows.

To be submitted to *Journal of Dairy Science*

After the breakout of melamine in infant formula in China and the previous contamination of gluten feeds in the USA, an investigation on the possible relation between different contamination levels of cow feeds and transfer of melamine in milk and cheese was evaluated. Being the levels of contamination close to the maximum permitted level in feed and food in Europe, and to the highest contamination of feed reported in USA and Europe, discussions were developed on the risk could arise from milk produced by cows fed those contaminated diets.

Third manuscript (Journal article)

Comparison of Ammonia and Tetra-Methyl Ammonium Hydroxide extractions of iodine in raw milk

*S*ubmitted to *Food Chemistry* on October 2, 2009.

The manuscript has the aim of comparing the efficiency and evaluating the uncertainty between two methods of iodine extraction: the official methods EN15111:2007 (that uses a tetra-methyl ammonium hydroxide extraction) and a more quickly and less expensive assay using an ammonia solution for iodine extraction. In both methods iodine was detected using inductively coupled plasma – mass spectrometry (ICP-MS).

Fourth Manuscript (Short Communication)

Speciation of inorganic iodine in raw milk using ion-chromatography coupled to ICP-MS

Not published

The most bioavailable form of iodine is iodide. The aim of the work was to settle an essay for the speciation of inorganic forms of iodine (iodide and iodate) in milk by ion chromatography coupled to ICP-MS. Since the milk samples were produced by cows fed three different level of iodine, another aim was to evaluate the distribution of inorganic form of iodine at different concentration of total iodine in milk.

2 Quality of milk: wealth and health opportunity

Milk is a fundamental component of human diet thanks for its complete profile of nutrients and related dairy products that can be processed from it. Unfortunately its production cost in energetic terms is quite expensive compared the important crops like soy and corn (Boland *et al.,* 2001). Initiatives, therefore, aimed to change the quality of milk products should complement and be consistent with existing methods of farming management in order to be cost efficient (Knowles *et al.,* 2006). Milk nutrient modification thanks to nutritional and genetic manipulations can represent the leitmotif for obtaining earning productions and customer satisfaction. As previously written, not all the nutrients have the same potentiality to be modified and above all, it is of paramount importance to understand if a modification of the milk composition is an advantage for the human nutrition without compromising the animal health and the cost efficiency. Apart from milk we have to consider that the diet is a mix of different type of foods, so a deficient element in milk can be for instance highly concentrate in vegetables, fishes or meats (lycopene in tomatoes, unsaturated fats in fish oils, iron and B vitamins in meats). With a comprehensive approach, prior to planning a feed fortification research, the nutritionist should therefore ask himself some questions:

Is an element surely correlated with human health or some specific disease prevention?

What is the bioavailable and physiologic active form of that element?

Is the element chemical form healthy or not for the animal?

What is the physiology and biochemical pathway of the element in the animal body?

Could the possible transferable amount of the element in milk modify milk or dairy products quality (make better or worse)?

Is that chemical source feasible for feed managements?

A clear summary about the possibility of minerals and vitamins for being modified by nutrition of animal was published by Swensson *et al.* (2007). After a review about the possible modification of milk composition by calcium, phosphorus, magnesium, potassium, sodium, chloride, selenium, iodine, iron, zinc and vitamins (A, E, K, B, C) the authors conclude as follows:

"The concentrations of Se and I could easily be changed in a favourable way by altering dairy cow diet. However, there is a risk of an excess of these two trace minerals, especially Se. Also, vitamin A and E show a strong correlation with daily intake: a higher concentration of roughage and pasture in dairy cow diets promotes these vitamins. Organic dairy production is probably favourable with respect to vitamins. Such a diet also promotes a well-functioning rumen and therefore also the synthesis of B vitamins. However, when altering the content of vitamins and minerals in dairy cow milk it is necessary to assess the impact on the overall milk composition"

According to the previous six questions and the bibliography studies, iodine and selenium seems to have the best scientific, economic and feasible evidence for being inserted in a quality improvement program of milk and dairy products by feed fortification. However, further studies could be necessary for a better understanding of the distribution of iodine and selenium form in dairy products and the effect of milk yield, species and breeds on their excretion from feed to milk.

The on-farm realisation of enriched-milk should be followed by commercial activities giving the product a friendly aspect for the consumer (Knowles *et al.,* 2006). It is clear that each investment in feed fortification would vanish if consumers do not recognize a plus-value to a particular enriched-milk or dairy product. Apart from the general knowledge about the consumer's determinants of choice, more interdisciplinary research and more studies based on a deductionist approach are needed to make real progress in the understanding of the determinants of eating and drinking behaviour and the prediction of food choice by the consumer (Koster, 2009). According to Cox and Bastiaans (2005) many consumers are not inclined to buy off-farm enriched-milk products because the general unsafe belief on food additives. In many countries some legal restrictions, particularly for infant-formula and primary foods, permit only unadulterated foods (Knowles *et al.,* .2006).

2.1 Methods for manipulating milk composition

Whichever method is used to modify the quality of milk composition is fundamental that it does not perturb other product features, like safety, shelf life, texture or taste. According to Knowles *et al.* (2006) the different methods can be classified into three principal branches:

Rumen modification

Genetic selection

Feed management

The nutrition in ruminants must consider the metabolism which can arise from the interactions between nutrients and rumen microbes. The rumen can represent an opportunity to improve animal productivity for instance through ruminal fibre and protein degradation, protein and amino-acids synthesis, and gas production (McSweeney *et al.,* 1999); on the contrary microbial fermentation are to be avoided, that is the cases of by-pass ruminal elements. The microbial pool can interact also with minerals and vitamins: dietary cobalt expedites the synthesis of ruminal vitamin B_{12}. The Co/vitamin B_{12} presence regulates the right production of methane, acetate and methionine synthesis, fundamental to avoid an over-production of succinate and the subsequent inhibition of propionate metabolism (Knowles *et al.,* 2006).

Some elements like calcium, phosphorus and magnesium have a strongly homeostatic regulation due to their importance in severe number of biochemical pathways in the bodies, and genetic selection is the most efficient method capable of altering their milk content (Davis *et al.,* 2001). The example of calcium generates immediate benefits to consumers and industries, but livestock selection is a lengthy process that could last 10 or more years, with the consequence of a not certain and easily foreseeable profit (Knowles *et al.,* 2006).

The diet of an animal is composed by different feed sources (fresh forages, silage, concentrates, supplements, salt-licks, yeast and water) on which it is possible to operate for changing the milk composition. In the lack of particular homeostatic regulation, the transfer of nutrients from feed to milk depends by the milk/plasma ratio concentration of the same element. This ratio represents the ability of mammary gland to accumulate elements into milk and to maintain a gradient concentration against blood plasma. The more the ratio is great the more an element offers good opportunities in feed fortification programs. The milk/plasma ratio for calcium is 12, which is much higher than those of iodine (about 1) and selenium (0.25), but these two latter have no homeostatic implication as for calcium and their final potential by feed is therefore effectively higher.

3 Adulteration of milk: economic damage and health risk

The ubiquitous environment pollutant such as dioxins, heavy metals, pesticides, veterinary drugs and polycyclic aromatic hydrocarbons are well known by farms and feed mill facilities. Their presence is practically inevitable, so the quality control actions are based on a limitation and reduction of the pollutant in feed and final animal productions. Our attention, instead, is focussed on the voluntary adulteration of milk and feeds to profit.

The adulterations of milk are addition of less expensive (and sometimes dangerous) substances to mask the lack of important features or ingredients: for instance the production of "Pecorino" cheese or cow buffalo "Mozzarella" with cow milk, the addition of milk powder in fresh milk. The Italian Ministry of Agriculture esteems that 20% of cheese production during 2004 in Italy was altered (Cunsolo, 2005), but despite the illegal aspect if we consider the consumer's safety concern, most of these adulterations do not represent a danger for the public health. The main dangers are on charge of the market and honest producers.

The effect of the melamine tainted milk ingestion on Chinese children was firstly published in September 2008: 54,436 children were involved, of which 12,892 were hospitalized. Four children died (Ministry of Health of the People's Republic of China, 2008). In the record of occurrences of adulterations the case of melamine has many particularities due to the chemical nature of the molecule, the worldwide market of melamine and urea, the link between feeds and milk. Regarding the chemical property of melamine, it is a triazine containing 66.6% in weight of nitrogen that is ideal to trick the Kieldahl evaluation of crude protein by the analysis of total nitrogen. The Chinese company Sanlu is considered responsible to have suggested their milk providers to lace low protein milk with melamine (Masters, 2008). A hypothesis to explain a possible reason behind the contamination of infant formula with melamine could be found in the United States Geological Survey - Mineral Survey Yearbook (2005), and in the Melamine Industry Update (DSM, 2007). Between 2001 and 2007 the melamine industry of China suffered over-production, contemporary melamine price froze and the cost of urea increased; therefore close to the period of infant formula contamination in China there was an exceed of melamine, plus a price increase of the material (urea) to produce it.

The link between milk and feeds is an interesting hypothesis carrying out by the contamination of gluten in China. As a matter of fact the contamination of melamine was registered around the world in many other foods, such as biscuits, candies, cakes and above all pet foods, because the roots of contamination were different batches of imported wheat gluten from China. Apart from the direct contamination of infant formula with the well known consequence in Chinese children, the high presence of melamine (about 10% in weight) in wheat gluten involved many countries and many industries due to the fact wheat gluten is a primary ingredient as thickener and binder in feed/food preparations. The unique health concern linked to melamine contaminated gluten was the death during 2007 of various dogs and cats in the USA, fed pet-food processed with that gluten (Dobson *et al.,* 2008).

Where is therefore the link between milk and feeds? Apart from gluten, feed mills produce numerous high protein products, which could be easily laced using melamine or urea. It is so possible hypothesise that ruminants could be accidentally fed contaminated ratios. Cruywagen *et al.* (2009) shows that the transfer efficiency of melamine from feed to milk in cows fed diet with 17.1 g/day of melamine is about 2%. This result is important because demonstrates the possible contamination of milk by milk not only directly but also via feeds. It is now important the engagement of research and industry for a better understanding of melamine pathway in animal, its carry over in products like milk, eggs and meat, and development of rapid analytical determination.