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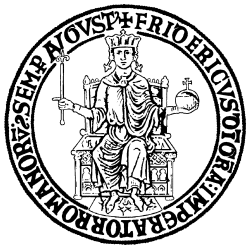
FACULTY OF VETERINARY MEDICINE



Doctorate Schools in

Production and Safety of Foods of Animal Origin

XXIV Cycle

BUFFALO BULLS FOR MEAT PRODUCTION:

FEEDING AND MEAT QUALITY

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1. GENERAL INTRODUCTION

1.1. The Buffalo farm

The world buffalo population amounts about 190 million of heads distributed as follows: Asia 96.4% (mainly concentrated in India, China and Pakistan), Africa 2.9% (with greater consistency in Egypt) and the rest in Europe (especially Italy, Romania, Georgia, Bulgaria and Turkey) and Latin America (Brazil, Venezuela, Colombia and Argentina). In the past 10 years, the buffalo population growth was around 20 million of heads and representing a growth of 12.5% (FAO, 2010).

In table 1 the countries in which FAO has information about Buffaloes population and the countries where there is knowledge to exist Buffaloes population, but in the FAO data base were not reported.

In 2008, buffalo, a triple-purpose specie (work, milk and meat), produced 89.2 million tons of milk and 3.5 million tons of meat. Between 1998 and 2008 the registered productions development was significant, considering that, milk and meat production have risen an increase of 43.47% and 16.32%, respectively. In table 2 the number of slaughtered and the relative increase in 45 years divided by continents were reported.

In the zoological scale the water buffalo is in the class *Mammalia*, order *Artiodactyla*, *Ruminantia* subordinate, family *Bovidae*, sub-family *Bovinae*, gender *Bubalus*, species *Bubalus bubalis*. This species is divided into two groups (Macgregor, 1939):

- *Bubalus bubalis* sp. known as “water or buffalo river” with 50 pairs of chromosomes bred in India and in western countries (live weight of adults: 500 and 1000 kg for females and males, respectively);
- *Bubalus bubalis* var. kerebau called “Carabao or swamp buffalo” with 48 pairs of chromosomes, present in the countries of Southeast Asia (live weight of adults: 350

and 650 kg for females and males, respectively).

The different breeding conditions between the groups (more intensive for river buffalo and more extensive for the swamp buffalo) have contributed to increase the morphological and productive differences between groups (Figure 1). The water Buffalo is sourced from the Asian buffalo, that is phylogenetically separated from the African buffalo (*Syncerus caffer*). The Asian buffalo originally included three different wild species: the Anoa of Sulawesi, the island of Mindoro and Arni Tamarai or Indian wild buffalo. On archaeological excavations in India evidences the presence of water buffalo 60,000 years B.C., but it is around 2000 B.C. that the domestication of the Arno (*Bubalus arne*) began in the Indus Valley (India) and through the centuries has given rise to the water Buffalo (*Bubalus bubalis*).

The Buffalo River breeds in the world are 18, and 16 of these are located mainly in the Asian continent (*Murrah, Nili-Ravi, Kundi, Surte, Meshana, Jafarabadi, Nagpur, Pandharpur, Manda, Jerangi, Kalahandi, Sambalpur, Bhadawari, Tharai, Toda and South Kanara*). The newest breed is the *Bufalypso* or *Trinitarian breed*, trained in Trinidad y Tobago islands. It is an intersection of four Indian breeds and has been primarily bred for meat production, however, in recent years has also improved in dairy production.

The present thesis is referred to the Italian Mediterranean Buffalo, originate from India, mainly bred in the south of Italy and selected for milk production.

In order to better describe the morphological characteristics of the Italian Mediterranean buffalo (Figure 2) the description of the ANASB (*Associazione Nazionale Allevatori Specie Bufalina*) has been reported (http://www.anasb.it):

- Head: harmonic, slightly elongated, with large sincipite ones in profile convex, covered with thick hair. Front short and broad with a convex profile accentuated in the male, broad nose and a long straight profile large ears and thick, carried forward opening horizontally, covered with short hairs and sparse long and abundant on the outside and inside. Big eyes, blacks, close, lively, mobile eyebrows and long eyelashes. Large mouth with strong jaws. Muzzle broad, black, with nostrils well developed and mobile.
- Horns: brown, symmetrical, 50-60 cm long in males and more in the females,

directed laterally and backward, the base's section is triangular in males and females oval, with transverse grooves and relief on the cranial face.

- Neck: a little bulky in the female, full of vertical folds, with slightly concave dorsal and ventral margin straight, convex and devoid of dewlap.
- Chest: strong and wide to help increase the thoracic cavity with skin fold in form of voluminous bag, more or less fleshy in the older animals of both sexes.
- Withers: extended, long and well arched, not too wide, with a raised median at the dorsal spinal of the dorsal vertebrae, more pronounced in males.
- Back: Long, broad, harmoniously blended with the adjacent regions.
- Hindquarter: harmoniously developed, tending to form a square. Slightly sloping to the rear, coccygeal and sacral vertebrae slightly raised but not high; the attack of the tail not returned.
- Tail: wide at the base, and rightly long.
- Thorax: wide and deep, harmonically merged with adjacent regions.
- Shoulders: strong and well-attached.
- Loins: broad, strong, aligned with the back.
- Abdomen: massive but not falling, fused with the thorax.
- Flanks: full and deeps.
- Limbs: short at the free portion and well-muscled, aplomb well-spaced. Garrett strong and wide, with a light angle; hoof tight and compact, soled high, especially at the heel; tethers short and strong.
- Coats and pigmentation: from light brown coat to the burnt brown, almost black, with deeper colour at the front of the trunk, sparse hair, long, more abundant in the free part of the limbs. Sometimes there are white hairs in front and at the end of the tail and socks in one or more limbs.
- Skin: colour slate gray or dark discoloured toward red at the skin folds, especially in the inside of the thighs and the insertion of the udder; muzzle, around the eyes, ears, anus, vulva, prepuce, scrotum and claws blacks.
- Udder: well formed, extended forward, soft textured, spongy, elastic, with thin skin,

unctuous and hairless, rich folds caudally after milking. Regular quarters and harmonious development. Long nipples, well-spaced, vertical, with large abdominal veins sinuous, with large fountains, mammary veins clearly outlined and visible.

1.1.1. *The evolution of buffalo farming in Italy*

The buffalo's presence in Italy has very ancient roots, as evidenced by the discovery of fossil remains in the Lazio region and in of Pianosa island (Tuscan Archipelago), probably dating back to pre-Roman period. Subsequently buffalo seems to be absent at the time of the Roman Empire, to reappear again in the sixth and seventh centuries AD. Until now it is unclear the period when buffalo was introduced in Italy, probably it was introduced during the Longobards period, with the barbarian invasions of the sixth century in 596 AD. According to other authors, the king of Normans spread buffalo throughout southern Italy, around the year 1000 as it was already present in Sicily by the Arabs. However, evidence shows that the buffalo is in Italy since the XII-XIII century. In particular, the buffalo was affirmed in Campania Region in the wetlands of the Volturno and Sele plains due to the inability to assign these lands for agricultural purposes.

Unofficial estimates, indeed, report that at the beginning of '900s the number of buffalo was between 15.000 and 20.000 heads and decreased after the II World War to 12.000 heads, decimated by the Germans in retreat after the landing at Salerno (Infascelli, 2009). According to the data reported by the National Livestock Register established by the Ministry of Health (www.statistiche.izs.it), in recent years, the Italian patrimony of buffaloes highly increased: from 81,684 heads in 2003 to 374,034 heads in october 2011, (Figure 3); most of which mainly concentrated in Campania Region (72.5 %) and distributed as showed in Table 3.

In the past, the buffalo was used as working animal primarily in irrigated areas and wetlands because it was the only animal with the ability to go and pull carts in this type of land (Zicarelli and Campanile, 2001); however, for its strong ability in adaptation, was also used for meat production. The breeding of buffalo for meat was carried out by landowners using the marginal lands for calves that are slaughtered at more than three years. Until few years ago, the farmers used for slaughter only very young animals or adults at the end of the productive career. In this way, two results has been acquired: in the first case, small amount of meat was obtained, in the latter case poor quality meat

was produced; moreover, the meat of the old animals, give off a strong smell of musk. In this context, it is as likely due to the relative frequency of renal disease in buffalo species that become worse with age (Roperto, 1979; Cortesi and Vaccaro, 1981).

A negative factor to consider in buffalo production is that the feed conversion index is lower than cattle when the comparison is made with beef cattle under farm conditions in Italy. This aspect causes the higher production costs.

The main source of income from Italian's buffalo farm is the marketing of milk to produce *mozzarella* cheese, suggesting that the activity is considered a risky because it is based on a single product. At the moment, the production of meat could be considered a sideline of buffalo dairy farming (Figure 4), or at least, an outlet for any market crisis. The meat sector development could represent an opportunity not only for the economy of Campania Region, but it's even important for all the areas where the buffalo are bred.

Another positive aspect to consider in favour of buffalo meat is that the skin has a more pronounced grain than cattle and thanks to the characteristic of the flower, the top of the skin, is more marketable, presenting an excellent resistance to abrasion and a good flexibility. No doubt that the development of such trade should be to decline all current disposal costs, besides the farmers could certainly be any income from the trade of the skin (Valvano, 2000).

1.1.2. *Meat consumption*

More than for other basic food groups in human nutrition, meat consumption patterns vary considerably over time, cultures and the personal situation. Besides complete or selective avoidance of meat intake for ethical or religious reasons, socio-economic factors and health aspects are likely to be the most prominent factors in determining meat consumption patterns (Mann, 2000; Holm and Mohl, 2000).

Linseisen et al. (2002) conducted a study in half million subjects in 27 centers across 10 European countries to obtain reliable and comparable estimates of dietary meat intake in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts. The results are shown in the table 4.

Early in the twentieth century in Italy the meat represented the 21,5% of food expenditure of households (INEA, 2004) but this percentage has steadily declined over the past 10 years. Like in the rest of the word, the meat represents one of the most

symbolic foods in terms of both socio-economic, cultural and its consumption has always been subject to significant changes, which are closely linked to different socio-economic scenarios that have occurred in the past in Italy.

After considerable growth in meat consumption that has occurred up to the 80s (ISMEA, 1993), linked to the increase in economic welfare, then there has been a stabilization determined mostly by dint of healthy and cultural. Moreover, there was a propensity to diversify consumption of poultry meat to the swine or that, at the expense of beef, with the exception of the calf, which was a product that is supported by a particular image (Baldi and Banterle, 2005).

Demand for meat, and especially the bovine sector, has continued to suffer significant disruption as a result of two incidents that occurred by the Bovine Spongiform Encephalopathy (BSE) in 1996 and, more recently, in late 2000 and early 2001. These events have disoriented consumers in Italy, as in Europe, resulting in the abrupt drop in purchases of beef that have impacted the entire production chain.

With regard to the buffalo meat, there are no official figures, however, is very simple to understand that consumption is very low. If we consider that the Italian buffalo patrimony with approximately 120.000 mares, and consider the parameters calving interval, reproductive efficiency, percentage of males at birth, mortality, and direct to slaughter all the males, you could count on a availability of buffalo meat amounting to about 130 g/head, which slightly affects the total 90 kg of meat that the average Italian consumes.

1.1.3. *Buffalo meat market in Italy*

In the past century, in Italy the buffalo meat market didn’t existe because this meat was not accepted by the consumer; therefore the farmers were used to slay the male buffaloes as born. Only the males born from high milk production buffalo cows were bred for the reproduction. In this situation only the females wich present productive or reproductive poblems were slaughtered, introducing in the market low quality meat and confirming the customer idea that buffalo meat was of bad quality (Borghese et al., 2010).

Therefore, in 1976 in the Animal Production Research Institute (Roma) a series of trials were performed to understand the preferable age and weight to slaughter buffalo calves

could in order to obtain good quality meat, in comparison with Friesian calves bred in the same conditions of feeding and environment. Different trials were effected, slaughtering buffalo and Friesian calves at 20, 28, 36, 52, 64 weeks of age, measuring performances *in vivo* and the carcass and meat quality. In the same time at University of Naples Federico II, different researches were effected on the possibility to rear males buffalo for meat purposes. In 1994 the FAO Inter- Regional Cooperative Research Network on Buffalo organized in Paestum (Italy) the International Symposium on buffalo products were meat eating quality were also reported, as in that time many farmers used to rear buffalo calves or young bulls with different diets and a market of buffalo meat and typical Italian meat products as bresaola, sausages, salami was going to be created. In 2009 the Regulation to produce the "*Carne di Bufalo Campana*" (Buffalo meat from South Italy) with P.G.I. (Protected Geographical Indication) was effected, similarly as for Buffalo Mozzarella Cheese, creating a meat market of high quality. Therefore now the best production quality is young bulls of 400 kg of live weight or more, obtained at about 15 months of age and it is very important to achieve the best diet in the fattening period, the last before slaughtering.

At present Italian breeders are trying to produce high quality meat for the luxury market (restaurants and gourmet food) adopting a production protocol in accordance with the P.G.I. symbol "*Carne di buffalo mediterraneo*" (Mediterranean buffalo meat). The geographic territory where the buffaloes are reared is the same as the D.O.P. "*Mozzarella di bufala Campana*": Campania and the south Lazio Regions and parts of the Puglia and Molise Regions. The calves can be weaned with milk substitutes or by nursing bovine cows. Acoording this breeding procedure buffaloes can fed fresh or conserved (silages or hay) forages and meals or concentrates until they reach no more than 450 kg live weight in order to avoid an excessive fat deposition The daily gain must be between 800 and 950 g/d for young bulls in order to avoid sick animals As indicated by European Community rules each hormones treatment is forbidden. Four months prior to slaughter the use of corn silage and of particular feeding stuffs is forbidden in order to avoid bad flavour in the meat and the animals must be reared on slattered floors or on floors where the straw is changed each week to avoid the smell of urine and faeces. Stress before and during slaughter must be avoided in order to guaranty the meat quality.

Carcasses must be included in the medium and abundant classes for fatness and in the good and optimum classes for conformation. Ageing must be effected for nine days at

least and the quality characteristics of the *Longissimus dorsi* muscle must be: pH 5.5 5.9, intramuscular fat < 3 %, protein > 20 %, cholesterol < 50 mg/100 g, iron > 1.5 mg/100 g, and total mesophil bacteria< 105 units/cm^2. These regulatory measures guarantee the high meat products quality. In particular different meat products such as: *bresaola*, *salami*, and buffalo cheese rolls with *salami* or ham sould be industrially or artigianally produced. Therefore, the aim of the Italian market is to develop products of good nutritional and organoleptic quality.

1.1.4. *The milk and the mozzarella's cheese*

In 2007 the world milk production amount to 559 million tons (Table 5), including all species that can provide milk for human consumption. The high producer is India, with the 14%, and Italy is in the ninth place with the 2,71% of the world's milk production. Buffalo milk production in the same year was 83.6 tons that represent about the 15% of the world milk production in 2007 (FAOSTAT, 2011). Like in the total milk production, India is the big buffalo milk producer with the 67% and Italy is the seventh with 0.28%.

Buffalo milk production 2009, amount to 92 tons FAO (2010), 8.5 more tons than 2007 (10.17%) without consider statistics data it is known that in the last years several important Countries had substantially increased dairy production (i.e. Brazil, Argentina and Venezuela).

In certain developing Countries, such as India, Pakistan, Egypt and Nepal buffalo milk accounts for over 50% of drinking milk, while in Italy buffalo milk is almost mainly used for mozzarella cheese production (Zicarelli, 2004). Today there are several products that are made from buffalo milk, among which we mention smoked *provola*, *ricotta*, *caciocavallo*, *mascarpone*, *caciottina*, *stracchino*, butter, yogurt and ice cream.

Mozzarella is a fresh cheese of spun paste native of southern Italy, made with buffalo milk and is considered the quintessential product of the Campania region (Figure 5). In 1570 the term mozzarella was used for the first time in a cookbook by Scappi, one of the cooks of the papal court (Guadagno, 1990). *Mozzarella* has roots in a past so remote in time, its tradition has been passed down from the chess maker to chess maker, in families up to the present day and receive the mark of protection D.O.P. that is the

patent that in this time offer guarantee about origin, traceability and typicity of the product named "*Mozzarella di Bufala Campana*".

Very interesting is the information that the Consortium for the Protection of *Mozzarella di Bufala Campana* DOP presented in 2008: in 2007, about 84% of "*Mozzarella di Bufala Campana*" (MBC) produced has been for the domestic market and 16% in the foreign market. An interesting fact is that in Italy only 38% of MBC was sold in the DOP area, remaining 62% that was sold in the various Italian Regions. About the international market, 51% of exports go to the European Union (EU) Countries, the remaining 49% to countries outside the EU.

1.2. The buffalo, animal meat producer

1.2.1. *Performance infra vitam and post mortem of buffalo young bulls*

The meat production is the result of two biological phenomena which are closely interrelated and intertwined: the weight gain, or growth, that is the increase in body size and weight, and development that is the change of shape, body composition and muscle mass. The knowledge of the phenomena of growth and development is extremely important for the optimal choice of weight and age for slaughter, according to the rearing system and diet in which the animals are subjected. The value of a beef animal is measured by different indicators, the largest amount of product per unit of time, that is to say, most kilos of meat in the shortest time possible, higher productivity and better quality. The fundamental characteristics of an animal for meat production are: the body weight, the daily weight gain, the feed conversion ratio and the slaughter performance.

- The body weight (BW) has the trend of classic sigmoid curve: a phase of rapid growth from birth till about 9 months of age, followed by a slower growth rate to the live weight of 300-500 kg. On reaching the adult live weight has a deceleration until reach a maximum asymptotically.
- The daily weight gain (DWG) can be calculated by allometric or quadratic function, but can simply say that it is the kilos gained by an animal in a period divided by the number of days.

The conversion ratio of foods is intended as the quantity of dry matter (DM) or energy consumed per kg of growth. The high cost of food, which accounts for more than 60% of the total production cost, makes the conversion index particularly significant. This parameter gets progressively worse with increasing live weight of animals as a result of changes in body composition. The speed with which the animal reaches its full development is defined as earliness, which is closely related with the trend of the described parameters.

During the growth, the various tissues grow at different times according to a precise order, during foetal life the nervous system development is particularly fast; during extra-uterine life nervous system is rapidly complited and the skeleton develop progressively. In the first stage of life also muscle tissue increases and complete its development, finally, fat tissue becaming to increase at the end of growth stage and could increase during full life. The speed of development of muscle tissue and accumulation of adipose tissue is affected by age, race, sex and the nutritional level of the diet. The effect of nutritional level, defined as net energy and crude protein in addition to maintenance requirements, it becomes evident by interacting with the gene for the development of muscle and adipose tissue and this is the reason why feeding plans should be taken into account the above factors. In particular fat deposition in the last phases of breeding could develop particularly fast in the more precocious specie. In the late subjects, however, it is possible to obtain higher gains weight as it is not necessary to reduce the level of nutrition to prevent too fat. In addition, individuals early reach their shape more suitable for the slaughter, "maturity", at lower weights than late.

In the Italian market, the consumers want to find meat with little fat infiltration. Another factor to consider in the feeding of meat animals is customers preference: e.g. the European consumers prefer meat with low fat levels; while in other Countries, as in South America, the customers give more importance to the flavour and taste characteristics. The recommendations are the following:

- In order to obtain meat with low fat level is preferable to administer in the last phase of breeding diet characterized by low energy levels;
- On the other hand to improve meat palatability and tenderness is preferable to increase fat deposition in the last phase of breeding, using diets with higher energy and protein levels.

The *post mortem* performance of the animal is the relationship between the live animal weight and total carcass weight, it is taken into account for its evaluation, the weight at empty stomach, hot carcass weight and carcass weight after maturation at +4°C.

Talking about international research on buffalo nutrition related to its ability as a meat producer, in particular the increase of the body weight gain, we should underline that the results are often discordant between each other and do not lend themselves to a fair comparison due to the large differences among genotypes, environment and farming techniques utilised around the world; this mean that this kind of research is only of locally interest.

However, world-wide, the growing request for animal proteins for human consumption has given rise to the potential improvement of buffalo meat production. The market interest in buffalo meat has progressively increased also in the developed countries, like Italy, due to the negative trend towards bovine meat consumption, which has worsened due to the BSE scare. However, until few years ago only very young surplus male calves, and buffalo cows or bulls at the end of their productive career have been slaughtered. In the first case the quantity of meat was scarce, in the second the poor quality gave little market value to this product. In the following chapters, the results of researches effected on buffalo as meat producer as well as concerning suggestions for different growing phases will be reported.

1.2.2. *Buffalo calves feeding*

The success of buffalo breeding depends on good management and balanced feeding of calves, which ensure the optimum growth rate so they can attain early maturity weight.

Colostrum

Aside from its nutritive value and for laxative action allowing the evacuation from the intestine of faeces and meconium (Arora, 1988), colostrum intake in newborn ruminants is important, because it is the main way to obtain maternal antibodies (Bogin et al., 1993). Indeed, a significant amount of immunoglobulins reach the blood of calves thanks to the permeability of the epithelial cells of the small intestine and the lymphatic

system (Stanley and Bushm, 1985). Calves start sucking at 2-3 hours after birth, and the absorption of immunoglobulins lasts up to 24 hours. This period is critical since many diseases of newborn calves have been shown to be related to insufficient colostrum intake and/or poor colostrum quality, which is determined by the content of immunoglobulins (Matte et al., 1982). The IgG1 is the best represented immunoglobulin in the colostrum and its passage from the blood begins four to six weeks before birth, allowing a concentration at first milking 2-10 times more than in the serum of maternal blood. According to Salerno and Tiberio (1963) and De Maria Ghionna et al. (1987) the concentration of immunoglobulins in the colostrum of Mediterranean Italian buffalo cows shows a rapid decrease from 74% of the protein content at 4 hours after the delivery to about 24% after 126 hours, with a corresponding increase in lactoalbumins and lactoglobulins. The immunoglobulin values reported by Salerno and Tiberio (1963) were 84.9 mg/ml at 4 hours, 31.4 mg/ml at 40 hours and 12.9 mg/ml at 5 days. De Maria Ghionna et al. (1987) found slightly lower immunoglobulins concentrations (61.8 mg/ml at 4 hours, 27.6 mg/ml at 48 hours and 5.8 mg/ml at 5 day) similar to the values reported by BargHava and Balakrishnan (1978) for Indian buffalo cows (68.7 mg/ml on the first day after delivery; 23.7 mg/ml on the second day and 1.01 mg/ml on the fifth day of milking). To ensure the passage of significant immunoglobulins, the intake of colostrum suggested for bovine calf is equal to 70 ml/kg of body weight (7%) at the first feed and 50 ml/kg (5%) at the next three (Gay and Besser, 1991). In the researches carried out on buffalo calves approximately 1.5-2 liters of colostrum are administered within 2 hours of birth and up to 6 liters/day in the first week of life (Arora, 1988; Romita and Dias Da Silva 1975 and 1978; Romita et al., 1977). Barghava and Balakrishnan (1978) reported 29.7 mg/ml of immunoglobulins in the blood of buffalo calves on the first day of life and an increase (35.7 mg/ml) on the second day. Similar values have been found on Mediterranean Italian buffalo calves: 4.2 g/l at birth and 32 g/l after one day of colostrum intake (Lombardi et al., 1996). Researches carried out on dairy cows reported an increase in the IgG1 concentration in the colostrum as the pregnancy number increased, reaching the maximum after the third delivery. The IgG1 concentration is significantly lower in the colostrum of animals that have had a continuous flow before delivery and of animals with no more than 30 days of dry period. The latter is unusual for buffalo, but the colostrum has to be discarded and not administered to calves. Severe malnutrition of the mother before delivery results in a marked decrease in immunoglobulin content in the colostrum. Gay and Besser (1991)

suggested avoiding the intake of colostrum obtained from animals with BCS lower than 3 at delivery. Pero et al. (2001), with a view to creating a colostrum bank to ensure the best immunoglobulin transfer during the best absorption period (24 h after calving), found that GGT is an accurate indicator of colostrum quality. Indeed, GGT activity and IgG content are highly correlated; in addition GGT measurement in dry chemistry is a very simple method.

Management

In order to avoid a high mortality rate in calves the most technically advanced farms are equipped with “delivery paddocks” where buffalo cows have to be moved about 20 days before the delivery (Alexiev, 1992; Ferrara and Intrieri, 1974a). Otherwise, calves are born in open air paddocks where manure tends not to be regularly cleaned; these conditions can cause not only low levels of vitality leading the calf to delay colostrum intake, but also omphalophlebitis the main cause of septicaemia. On dairy buffalo farms, due to the high price of buffalo milk, newborn calves are separated from the mother at birth or within 4 hours (Arora, 1988; Alexiev, 1992). The separation can create failure in milk secretion due to the keen maternal instinct of buffalo (Arora, 1988) but otherwise the calf could subsequently refuse the artificial teat (Ferrara and Intrieri, 1974b; de Franciscis and Zicarelli, 1974). In Italy in the past, in order to milk buffalo cows whose calves have died, the hide of a dead calf was placed over another calf, tricking the mother with its familiar odour. In some farms milking parlours were created with a box for a calf placed near each stall. In the last few decades, after a period in which oxitocin was widely used to stimulate milk secretion, the problem has been overcome as the calves are fed artificially; once they reach maturity, they accept being milked by man.

During the administration of artificial milk, buffalo calves are placed in individual cages and subsequently in stalls, equipped with or without external pen. The former, made of zincate metal and fitted with a manger divided into one section for hay and concentrate and one section for a pail of reconstituted milk, is easy to keep clean and reduce diseases spread through contact, the main cause of mortality in calves less than 4 months hold (Salama, 1995). On the other hand, individual cages are expensive, cumbersome to store and need time to clean. Their number on a farm depends on the type of milk substitute utilised; according to Zicarelli (1990) buffalo calves have to stay

in the cage until weaning if fed hot milk substitutes, for less than 10 days in the case of acidified milk. In both cases, the calves are subsequently placed in the stalls, grouped according to age or live weight.

Both under-feeding and over-feeding are harmful, the former leading to poor growth rate and the latter from a disease point of view. Over-feeding of milk usually causes undue fermentation in the large intestine and helps pathogenic organisms to multiply; this affects the intestinal wall, leading to enteritis (Arora, 1978). It is advisable that the first milk administration occurs when the calf has expelled the meconium and is hungry.

Artificial feeding can be carried out using bottles or pails fitted with rubber tubes, both slowly administering small quantities of milk, in order to determine the correct functioning of the oesophagus groove. Feeding from a pail requires time to educate the calf - it can be helped by having one or two fingers inserted into its mouth to stimulate sucking - for the first administration that is dangerous because the calf might not receive a sufficient amount of colostrum. On the contrary, once the calf is used to drinking from the pail, it can intake large amounts of liquid resulting in an altered response of the oesophagus groove. Feeding from the bottle with a teat is undoubted more natural even if cases of *ab ingestis* bronchopneumonia are not rare when calves are forced to use a teat. A combination of the two methods is represented by the pail fitted with one teat for calves kept in cages or more teats when groups rationing is used in the case of cold acid milk replacers. The last innovation for milk replacers distribution consists in computerized milking. It recognizes the calves thanks to a microchip applied to their auricle of the ear and places the daily ration at their disposal divided into several meals. Computerized milking records the individual milk intake, rapidly detecting decreasing intakes often related to a precarious state of health.

Milk replacers

The milk replacers for bovine calf feeding initially consisted of skimmed milk powder and milk serum with additional animal (butter, suet) or hydrogenated vegetable fat. Subsequently with the increase in milk and casein prices, soya, distillers' products, brewers' dried yeast, alfalfa, fish and meat have been used as a source of proteins. According to Roy (1984) and Petit et al. (1989) the "non-casein" protein substitutes determine an increase in abomasal filling resulting in a reduction in protein digestion in the abomasum and small intestine. In particular, the milk replacers with soya or its by-

products seem to be responsible for decreased HCL, rennin, pepsin, trypsin and chymotrypsin secretion. Furthermore, the antigenic power of soya should cause a shortening of the intestinal villi and variations in abomasal and intestinal motility, resulting in diarrhoea. According to Lalles et al. (1995), a decrease in antigenic activity could be obtained by using hydrolysed isolate soya proteins.

The protein content of milk replacers usually ranges between 19% to 27% DM, while fat content between 10% to 25%. Using the so-called “high fat replacers” whose fat content ranges between 20% to 25%, the calves perform better and the incidence of diarrhoea decreases; by contrast diarrhoea occurs frequently using the “low fat replacers” (fat less than 20%) due to the high content of lactose (Wijayasinghe et al., 1984).

The milk replaces are produced either by the spray or the rolled method and homogenised in order to increase their digestibility; storage and the nutritive characteristics are influenced by the pH value (fresh milk: 6.6; milk replacers: 6).

In formulating milk replacers for buffalo calves it must be taken into account that buffalo milk shows a higher Ca/P ratio (Ca 1.8-2 g/kg; P 1.1 g/kg; ratio 1.73) than dairy cows milk (Ca 1.1-1.2 g/kg; P 0.8 g/kg; ratio 1.33) and that the intake capacity of buffalo is lower than that of a bovine calf (2% vs. 2.4-2.8% of DM/100 kg LW). To allow the same concentration per kg of milk reconstituted at 12-14%, 13-15 g of Ca and 8.5-10 g of P/kg of milk powder DM are required. The Ca/P ratio during suckling, due to the vit. D deficiency of milk, is the only factor which guarantees an optimum absorption of both minerals. The latter is very important given that buffalo is classified as a precocious species, reaching adult body composition rapidly with very swift conclusion of skeletal tissue growth. In particular, the pelvis bones assume shape and structure influenced in the first period of the extra-uterine life; it must be underlined that a non-harmonic development of the pelvis is recognized as a factor which can cause uterine and vaginal prolapse in buffalo cows and that this pathology hardly exists where natural suckling is prolonged until 7-8 months of age, whatever diet is fed during lactation or dry periods (Zicarelli, 2000). In addition, in buffalo milk also a lower Ca/protein ratio (0.35 vs. 0.42 in bovine and buffalo milk, respectively) exists, which suggests the function of Ca is not only to promote rennet coagulation but also to satisfy other physiological requirements. According to Ferrara and Intrieri (1974b) the percentage of colloidal calcium in buffalo milk is about 80% of total calcium while in

bovine milk it is about 67% (Zhu Ping et al., 1996). Better use of colloidal Ca or a different requirement for buffalo cannot be excluded. Compared to bovine, buffalo has got a more developed skeletal apparatus which, as its specific weight diminishes, allows it to swim more easily in the water, which is why it is called “river buffalo”. In natural suckling conditions, the different intake capacity between buffalo and bovine calves, penalizes the former for proteins, lactose, Ca and P intakes (table 6). However the higher lipid intake enhances protein use in buffaloes, greatly diminishing its amount for satisfying energy requirements. Because no significant live weight differences exist between the two species, and the skeletal apparatus has a greater incidence in buffaloes, it can be assumed that the lower Ca intake in buffaloes depends on a better capacity of absorption due to the higher Ca/proteins ratio in buffalo milk. Comparing the amounts of colloidal Ca intake/100 kg LW, differences are minimal and advantageous for buffaloes.

Milk replaces for buffalo calves must be integrated with no more than 5 mg/kg of copper; Zicarelli et al. (1981) reported episodes of intoxication with high death rate in buffalo calves fed milk replacers integrated with 20 ppm/kg DM of Cu and 92 ppm/kg DM of Fe. Probably the unbalanced ratio between the two microelements causes the pathology which does not occur when buffalo calves are fed milk replacers integrated with 30 ppm/kg DM of Cu and 240 ppm/kg DM of Fe.

In recent years, use has been made of milk replacers consisting of dried milk sera, serum proteins and animal or vegetable fat (so-called acid milk), easy to mix in water at room temperature. They are produced in the medium acid (pH about 5.7) and high acid (pH about 4.2) forms. The first can be prepared with skimmed milk and their storage is about 24 hours. The high acid form keeps longer but the complete lack of caseins (milk serum is the best protein source) makes them suitable only for calves three weeks old (Webster, 1984). One of the main advantages of acid milk is that it can be fed by grouping the calves from 7-10 days of life, thereby considerably reducing the time spent on administering feed. Due to *ad libitum* feeding and the low temperature of milk, the calf frequently ingests and in small amounts, resulting in beneficial effects on digestion. The diarrhoea incidence decreases due to the bacterio-static action resulting from the low pH. On the other hand, the higher milk intake leads to high feeding costs. Milk replacers which are most frequently used for buffalo calves can be distinguished into two great families: those containing 60% milk, and so having about 45% casein, and those without milk and casein, prepared essentially with serum-proteins. The former

ones give better results. In table 7 the chemical composition of an acidified milk replacer for buffalo calves is reported.

Many research have been effected in order to substitute buffalo milk with replacers without affecting the growth and welfare of calves. According to Arora et al. (1974) complete substitution results in a significant reduction in the growth rate. Abou-Hussein and Raafat (1962) found a lower (18% less) growth rate in buffalo calves fed bovine milk compared to buffalo milk.

In Italy many studies have been carried out on milk replacers and on pre-weaning techniques for the purpose of producing buffalo meat from young animals. Since 1963, the first systematic research on the artificial suckling of the buffalo calf has been conducted at the Zootechnic Institute (today Department of Zootechnic Science and Food Control, section B. Ferrara, DISCIZIA) of Naples University, Faculty of Veterinary Medicine.

The research was effected on male buffalo calves, after colostrum administration, which were raised until the weight of 150 kg. The use either of cattle milk or of a milk replacer was efficient in terms of physiological aspects and proved to be very profitable. Compared with buffalo milk, employing cattle milk and milk replacer yielded savings of 28% and 60%, respectively. In order to grow 1 kg of live weight, the animals required 6772, 7084 and 7875 calories, using buffalo milk, bovine milk or milk replacer, respectively. The employed milk replacer contained a higher lipid concentration compared to that usually administered to bovine calves. Daily weight gains and slaughter yields registered in buffalo calves fed with milk replacer were 0.973 kg/59.28% *vs* 1.103 kg/59.40% and 0.987 kg/59.06% obtained in the groups fed with buffalo or cattle milk, respectively (Ferrara et al., 1964). The chemical and bromatological characteristics of meat were similar in the different groups. In addition, the quality was always good: the meat was judged to have very appreciable tenderness, flavour and juiciness according to subsequent trials (Ferrara et al., 1969).

Similar results on the growth index of buffalo calves in the first months of life were reported by de Franciscis et al. (1970). Employing a milk replacer with a fat/protein ratio similar to that of buffalo cow milk, Romita and Dias da Silva (1975) obtained the same daily weight gains in male (0.919 kg/d) or female buffalo calves (0.933 kg/d) until the final weight of 220 kg (table 8).

Tripaldi et al. (2001) compared the performance of three groups of male and female

buffalo calves fed acid milk replacers for bovine (group 1, concentration: 12.5%) or for buffaloes (group 2, concentration: 18%; group 3, concentration: 22%). Calves, which had free access to hay and concentrates, were fed milk replacers *ad libitum* until 4 weeks old; subsequently the amount decreased 1 litre per week until the 8th week and, starting from the 9th week until weaning (effected when calves intook at least 1 kg of concentrate and/or when birth live weight doubled), 3 liters/head/day were administered. The trial was divided into three experimental phases: A: *ad libitum* administration; B: rationing of milk replacers; C: beginning of hay and concentrate intake. The initial weights of groups were different due to the fact that calves were born during a two-months period and they were assigned alternatively to the different groups, also according to sex. The intake of milk replacers was significantly higher for group 1 decreasing as its concentration increased; the intake of milk powder was the lowest for group 1 and highest for group 2 (0.75, 1.09, 1.01 kg, for groups 1, 2 and 3 respectively). As reported in table 9, the daily weight gain (DWG) was significantly lower for group 1 during the A and B phases, reaching the values of other groups during the third phase. In any case, considering the whole trial, group 1 showed the worst results. Group 3 had the most favourable DWG even if the differences with group 2 were not significant. The feed conversion index (FCI) during the phase A was significantly lower for group 1 (1.86, 1.29 and 1.19, for group 1, 2 and 3, respectively) and also during the whole trial. The milk replacers for buffalo at 22% of concentration showed the best results.

Due to the European Community law (EC Reg. 2821/98) which forbids the use of antibiotics for the milk powder preparation, Roncoroni et al. (2001a) carried out a research to ascertain the influence of milk yeast addition to the milk replacers on growth performance, health and the metabolic profile of buffalo calves. The subjects, 7 days old, were divided until weaning into three groups R (fed hot milk replacer), F (fed hot milk replacers with an additional 20 g/head of lyophilized milk yeast only for 5 days after colostrum intake) and Y (fed hot milk replacers with an additional 125 ml/head twice a day of a yogurt prepared by dissolving the same lyophilized yeast in buffalo milk). The calves of groups F and Y showed a lower incidence of respiratory problems, confirming the efficacy of probiotics to prevent these pathologies. Group Y showed a higher incidence of naturally resolving gastro-enteric pathologies and better hydration of skin and mucosae confirmed by the lowest values of hematocritus (Roncoroni et al., 2001b). These conditions positively influenced the growth performance of group Y,

which was significantly ($P<0.05$) higher than those of group F (DWG: 0.75 kg vs 0.61). In any case group Y showed better growth performance also compared to group R (DWG: 0.73 kg), demonstrating the efficiency of milk yeast as an alternative to antibiotics.

Failla et al. (2001a), aiming to obtain high quality meat from buffalo calves fed only milk until slaughtering, compared two types of milk replacers: the first containing 60% of skimmed milk (group R), the second based on cereals and soya (group V). The milk replacers were fed for 3.5 months at 18% concentration (phase C), subsequently for 2.5 months at 20% concentration (phase F). As reported in table 10, during the first phase group V showed significantly lower intake of milk replacers, due to its lower palatability, resulting in 30% of DWG. Even if the subsequent DM intake of group V was higher, the total DWG was higher for group R. The dressing percentage, and the conformation and fat scores were higher for group R, which also showed more favourable carcass composition. On the other hand group V showed significantly higher incidence of fore quarter and better water holding capacity (0.92% vs 0.76%, for group R and V respectively; $P<0.05$) (Failla et al., 2001b).

Weaning

Usually bovine calves are fed milk up to 55-60 days; starting from 30-40 days, the amount of milk is halved and the calves are fed once a day or with diluted milk, in order to encourage the intake of solids. The latter practice should not be adopted before the calf has begun to intake the starter concentrate. However some farmers wean bovine calves less than 4 weeks old, mixing the concentrate with the milk.

Salama and Mohy El Deen (1993) showed that, by feeding buffalo milk (10% body weight), buffalo calves consuming 0.7 kg DM of solids/head/day could be weaned at 56 days of life. Ferrara et al. (1964) studied the pre-weaning of buffalo calves administering alfalfa hay and a pellet concentrate, starting from the third week of age. The results showed a significant intake of solid feed starting from the 40^{th} day of age, that rapidly increase until the complete weaning was achieved, in the second month of life. The buffalo calves raised until 6 months of age reached an average weight of 175 kg with a daily gain of 0.816 kg. For each kg of growth 2.70 UF were administered, giving a slaughter yield of 50.6% (table 11).

Nour and Tag El Din (1990) conduced an experiment on 24 male buffalo calves divided

into two groups fed with different starter (cooked and uncooked). Each group was divided into two sub-groups, the first starter supplement with 3.5 g of protected methionine and the second starter fed without methionine. The results showed that the cooked starter increased the daily gain and feed efficiency more than the uncooked. The addition of protected methionine significantly increased, daily weight gain (430 vs. 400 g in the first group; 410 vs. 340 g in the second group) in every case.

Palladino et al. (1993), effected two subsequent studies: in the first trial, the effects of two intake levels (825-842 vs. 935-936 g of DM) of acidified milk replacer and of two administration periods of buffalo milk (<10 vs. 20 d) on the intake and growth rate of 53 buffalo calves (also fed with hay starting from the 15th day and with concentrate from the 30th day) from 10 to 60 days of age were evaluated. Dry matter intake depended on the dry matter content of acidified milk replacer and not on that of hay plus the concentrate. Feeding costs were higher for calves fed for 20 d with buffalo milk than for those that had the highest level of acidified milk replacer DM intake; the latter also showed a higher weight gain (670 vs. 614 g). In the second trial the effects of weaning begun at 45 d (with a reduction of 31% of acidified milk replacer DM) were assessed. In the 10-60 d age period, this technique decreased daily feeding costs but did not reduce the cost of weight gain. Dry matter intake until 45 d of age depended on DM of the acidified milk replacer; in the 45-60 d period the calves given less acidified milk replacer showed a daily intake similar to that of the controls. Between 60 and 80 days of age the subjects that were weaned gradually transformed dry matter better than subjects weaned brusquely at 60 days.

Di Lella et al. (1998), in a trial aiming to verify the influence of the feeding programme on growth dynamics of young buffalo bulls, divided 24 seven-days-old buffalo calves old according to a 2 x 2 factorial design: two weaning ages (63 vs. 84 d); two weaning concentrates (CP 17 vs. 14%; starch 37 vs. 29.6%, as fed). The calves of groups A and B, weaned at 63 d, received until 42 d 6 l/head/d of acidified milk replacer, in the ratio of 180 g/l of water. Subsequently, the replacer amount was gradually decreased, administering the same volume. The animals of groups C and D received, until 56 d fed 8 l/head/d of the same milk replacer, but in the ratio of 140 g/l. Also in this case, leaving the volume unchanged, the replacer was gradually decreased. Roughly chopped alfalfa hay and weaning concentrate were available from the fifth week; corn silage was administered starting from 70 d. After weaning, the animals were fed *ad libitum* hay and corn silage; the concentrates were administered in the amount of 12 kg/d/group. The

weaning age did not influence performance. By contrast, the weaning concentrate strongly affected the growth dynamics in the first 6 months: in this phase the concentrate with higher protein and starch content had better results. However, in the subsequent period (6–16 months) because of compensative growth, improved performance was obtained using concentrates with less favourable characteristics. These observations suggest that concentrate with higher protein and starch content can be administered during the weaning, when the calves are destined to be slaughtered at lower weights compared to those reached in this trial (400 kg); otherwise it could be opportune to administer the protein-poor concentrate in order to contain feeding costs. Esposito and Di Palo (1997) reported a comparison between two groups of buffalo calves, both weaned at 60 days that, starting from 45 days, received 0.617 kg DM of milk replacers + hay and starter concentrate *ad libitum* (group T1) or 0.416 kg DM of milk replacers + hay and starter concentrate in a ratio of 25:75. In the 10-60 day period the DWG of group T1 was significantly higher (LW at 60 days: 75 kg vs. 64 kg). In the post-weaning period (60-80 days) the DWG was higher for calves of group T2 (0.701 kg vs. 0.471 kg) since they where able to intake 1 kg of starter concentrate for the 60th day while group T1 reached the same level of intake on average 5 days later. However, it was not advisable to decrease drastically the milk supply at 45 days, because buffalo calves of group T2 were in any case underweight and needed a long time to overcome the shortfall occurring during the first 60 days of life. Although the drastic reduction of milk administration at 45 days allows an increase of concentrate intake, inadequate rumen development can result in a less efficient feed utilization with clear deficiency signs such as shaggy hair and/or disharmonious body development.

Cutrignelli et al. (2003a) carried out a study on 14 buffalo males divided into two groups, aimed to bring forward the weaning age and thus reduce the administration of milk replacer. Subjects of group A (weaned at 30 d) from birth were housed in individual boxes and fed until 15 d according to husbandry practice: colostrum in the first day, 4 l/head/d of milk replacer, in the ratio of 200 g/l of water subdivided into two meals a day. From the 16th day of life the milk replacer administered was gradually reduced by 1 litre every 5 days. From the second week the subjects received chopped (1-10 cm long) rye-grass hay and weaning concentrate (M1: CP 14.5% and 0.91 UFV/kg as fed). From 51 d of age, animals were transferred ia a collective box and the weaning concentrate was replaced by starter one (M2: CP 14.9% and 0.9 UFV/kg as fed). Group B was reared as follows: housed in individual boxes for the first 3 weeks of

life, subsequently in a collective box. The animals received 4 l/head/day of milk replacer until 35 d, after which the quantity was gradually reduced by a litre every 5 days to be weaned completely at the age of 50 days. When the subjects were removed into the collective box they began to receive M2 concentrate and rye grass hay *ad libitum*. From the age of 51 days both groups were raised in the same way. Daily individual consumption (g/d) of M1 concentrate for the A group varied considerably (1-15 d: 96.61 ± 88.69; 15-31 d: 226.4 ± 95.26; 31-51 d: 834.63 ± 237.81). In the subsequent growth stage, the hay and M2 concentrate daily consumption for group A were 3.02 and 9.51 kg/d, respectively. Hay consumption in group B was slightly higher (3.41 kg/d) than that of group A, although group B recorded a much lower concentrate intake (6.89 kg/d). Group A live weights and weight gains in the different periods were lower than those in group B, although the differences never being statistically significant. In the first 3 months of age all the subjects had low weights and weight gains, due to the scarce quality of hay. Successively (Cutrignelli et al., 2003b), they showed a compensatory growth and the performances were improved (DWG in the period 180-210 d of age: 963 ± 158 vs. 839 ± 109 g/d, for group A and B, respectively). In the light of the above research, the weaning of buffalo calves should depend on live weight; according to Zicarelli (unpublished data) the best moment is when the calf reaches 75 kg LW. This means that feeding costs for raising buffalo are higher than those for bovine calves. The amount of hay and concentrates consumed before 45 days appears insufficient to significantly reduce the intake of milk replacers, which is more expensive. Ideal management comprises the formation of groups of 8-12 weaned calves of the same age and weight with at least 3 m^2/head of space and fed, until 3 months old, 1-1.5 kg of starter concentrate plus total mixed ratio (TMR) and long hay *ad libitum*. The chemical composition of the TMR (consisting of corn silage, hay and concentrate) should be the following: CP 150 g, NDF 400 g UFL 0.9, on dry matter basis. Between 3 and 8 months of age, the administration of starter concentrate can be suspended and the young buffalo can be fed 3.6 kg DM of the above described TMR plus hay *ad libitum*.

An innovative method to obtain “biologic” young buffalo bulls could be the cow-calves system adopted in Brazil (Campanile et al., 2001). As reported in table 12, due to the high price of buffalo milk, the utilization of dairy cow at the end of her productive career to wean two buffalo calves should be financially more favourable. This should produce economically more favourable results also compared with to the administration of milk replacers, thanks to the compensation for the nurse by the EU. Because of

sanitary reasons (inter-species transmission of infective diseases) the system should be utilised in stalls outside dairy farms.

1.2.3. *Buffalo growth performances*

In Italy, many researches have been effected aiming to individuate the best age and weight for slaughtering, whose results are summarized in table 13 (Ferrara and Infascelli, 1994).

Buffaloes reached the average weight of 338 kg in 1 year; between 6 months -1 year the daily weight gain was 0.904 kg, the slaughter yield was 52.70% and the meat/bone ratio was 4.50. Compared with bovine calves at the same age, buffaloes presented a strong negative influence of hooves and, particularly, of skin (2.8% and 13.5%, respectively). Both neutered and unalterated subjects were utilized to produce buffalo weighting over 400 kg feeding rations rich in cereals and poor in crude fibre (nutritive value: 1.84 UF/100 kg of live weight). The weight of 440 kg was reached around 16 months of age with an average daily gain of 0.883 kg for the entire vs 0.851 kg for the castrated. To grow 1 kg of live weight the entire consumed 5.59 UF while the nutered consumed 5.67 UF. The slaughter yield was higher for the neutered (57.83% vs 56.77%) due to the lower weight of the hooves and, particularly, of the skin. Hence, the castration seems to cause a "refinement" for buffalo as it does for bovine.

Further investigations aimed to compare the meat production of buffalo and Fresian males feeding both hay *ad libitum* and concentrates have been effected (cited by Ferrara and Infascelli, 1994). The synthesized results were:

- similar weight gain (0.987 vs 0.949 kg/day, for bovines and buffaloes, respectively);
- higher dressing percentage for bovine (60.5% vs 56.6%);
- similar percent of meat in the carcasses (about 62%) due to the lower bone incidence (16.9% vs 20.2%) but higher fat content (21.2% vs 18.1%) in buffaloes. The adipose tissue distribution was different: buffalo carcasses show lower intramuscular but higher separable fat content.

Successive studies effected comparing buffalo and Fresian males both fed corn silage, alfalfa hay and concentrate for a 1.1 kg/day of weight gain, demostrated a lower growth

rate in buffalo until 14 months of age (0.97 kg/day). The buffalo carcasses were lighter with lower bone but higher sub-cutaneous fat content.

Di Lella et al. (1998) compared the performances of 3 groups of male buffaloes fed isoenergy and isoprotein diets (UFV kg DM 0.84, CP 14.5% between 6 and 12 months; UFV/kg DM 0.81 and CP 12.1% after 12 months of age) constituted by different ingredients. The best DWG (kg 0.887) were obtained for subjects fed diet with at least 50% of corn silage; however when large amount of this feed is not avalilable, the authors suggest to utilize ammonia treated straw, due to the high cost of hay.

In order to increase the knowledge about buffalo growth, characterised by periods of very scarce weight gain followed by compensatory phases, Infascelli et al. (2001) carried out research on 12 six-month-old male buffalo, equally divided into group A (111.0 ± 6.9 kg LW) and B (116.7 ± 7.6 kg LW). Group A was fed 100 g DM/kg MW of diet (CP/DM 14.1%, UFV/kg DM 0.84) based on rye-grass silage, rye-grass hay and concentrate. Group B received the same diet, but every 2 months the DM was alternatively 20% more or less than group A. From 300 kg LW until the end of trial (477 d of age), group B received diet (CP/DM 14%, 0.88 UFV/kg DM) obtained by varying the proportions of feeds. Between 180-238 d of age, group B showed a higher daily weight gain (DWG: g 480 ± 0.46 vs 510 ± 0.34, for A and B); however the conversion index (CI) was worse due to the higher amount of DM and UFV intake. In the interval 238-303 d, with a decreasing DM intake, group B showed lower DWG and worse CI. In the following interval, notwithstanding the increase in the DM intake, group B had similar results. On the contrary, between 380-415 d, group B had more favourable CI (UFV/kg weight gain 5.195 vs 4.452 for A and B) with slightly lower DWG (g 1143.0 ± 0.28 vs 1023.0 ± 0.14, for A and B). Group A showed the best results for the period 180-415 d, even if differences were not significant. Increasing the energy density of the diet in the interval 415-477 d slightly improved the total (180-477 d) DWG of group B, however less than that of group A (855.2 ± 0.58 vs 769 ± 0.25). Consequently, the total CI of group B was worse than that of the interval 180-415 d. Considering the whole trial, the buffalo growth curve showed a progressive increase in DWG, reaching its peak at 303 d of age (180 kg LW) until 415 d (300 kg LW). Successively the CI considerably worsened.

In order to evaluate the influence of diet nutritive level on the perfomances and on meat quality of buffaloes weighing 400 kg L.W. (Amante, 2003), twelve Murrah female

buffaloes were equally divided in two groups fed diets with high (H group: 6.95 kg of DM; 0.83 UFV/kg DM, 12.6% CP) and low nutritive level (L group: 5.43 kg of DM; 0.66 UFV/kg DM, 14.1% CP) for 29 weeks. Group L showed a BCS significant ($P<0.05$) decrease starting from the seventh week; significant differences of BCS were recorded between groups along all the experimental period (4.25 vs 3.38, for group L and H, respctively; $P<0.01$). At the end of the trial, group H showed an increase of 50 kg LW, while for group L, LW resulted unchanged, notwithstanding the energy intake in this group was not sufficient to meet the maintenance requirements suggested for buffalo in temperate-continental areas (VFU: 1.4 + 0.6 x q LW). According to the author the energy requirements for maintenance result lower in tropical climate areas; in addition it is possible that in conditions of diet energy deficiency, when the protein requirements are met, the nutrients utilization results more efficient (Campanile et al., 2001). At slaughtering, the dressing percentage resulted significantly higher for group H (54.9 vs 49.3%; $P<0.05$), while the meat chemical characteristics were superimposable between groups.

More recently, Mahmoudzadeh et al. (2007) carried out a study using 27 heads of 15 month buffalo male calves with initial live weight of 287 kg. The animals were individually housed and randomly allocated into 9 treatment groups of three animals each. Three levels of energy with three levels of crude protein were formulated to provide 90, 100 and 110% requirement equivalents for 900 g expected body weight gain. Dry matter intake was not significantly different betwewhen animals received medium energy diets. The dressing yield as well as the meat percentage was not affected by the type of the diet, but abdominal fat was significantly higher in medium energy diets ($P<0.05$). The authors concluded that the optimum fattening performance of 15 month old buffalo male calves may be obtained by providing around 10.42 MJ/kg of dietary metabolisable energy and about 10.22% of crude protein.

1.2.4. *Buffalo meat quality*

As regards the quality of meat, the results of researches carried out on buffalo are highly interesting. In table 14 and 15, the chemical composition and fatty acid composition of buffalo meat are respectively reported for 6 (Gigli et al., 1978) ad 15 month (Cutrignelli et al., 1996) old subjects. The aged animals show higher dry matter, protein, fat, and more saturated fatty acids, in particular C:16 and C:18, more C 18:1 and less C 18:2. In

each case all the data presented confirm that buffaloes meat have interesting nutritive characteristics both at 6 and at 15 months of age, in comparison with bovines.

The fatty acid profile and the cholesterol content of the food arouse high interest in human medicine due to their influence on the functionality of the cardio-circulatory apparatus. A number of epidemiological researches (Sinclair et al., 1982) put in evidence that diets with high content of saturated fatty acids (SFA) were associated with high levels of serum cholesterol (especially of low density lipoprotein, LDL) which appear important in atheroma.

The LDL carry most of the cholesterol in the plasma. It is generally accepted the theory that the increased levels of LDL has an etiological role in the onset of atherosclerosis and CHD. It seems that these lipoproteins, virtually harmless to its original state, become dangerous after an oxidation process that occurs in the liver. The process of developing atherosclerosis begins when the macrophages ingest modified LDL, contributing to their transformation into foam cells. The accumulation of foam cells in the intima results in the formation of fatty streaks. These do not produce significant obstruction of the artery, but are gradually converted, by a mechanism similar to the formation of scars in fibrous plaques, which in turn is gradually transformed into atherosclerotic lesions, responsible for most clinically relevant events. The process of oxidation of low density lipoproteins *in vivo* is not well known and is thought to be inhibited by the presence of plasma antioxidants such as ascorbic acid.

Ulbricht and Wheelock (1989) reported that:

- diets high in C18:0, stearic acid, do not raise serum cholesterol;
- short-chain SFA (C 10 and below) likewise do not raise blood cholesterol, so the putative atherogenic SFA are C12:0 (laurico), C14:0 (myristic) and C16:0 (palmitic). Myristic acid is the most atherogenic, with about four times the cholestrol-raising potential of palmitic acid (Hegsted et al., 1965).

Hornstra et al. (1975) and Renaud et al. (1986) showed that long-chain SFA accelerate thrombus formation whereas PUFA and MUFA do not, and that it is the longer-chain SFA (that is, C14:0, C16:0 and C18:0) which are thrombogenic. PUFA are considered protective factors: ω-6 fatty acids show mainly anti-atherogenic activity while ω-3 fatty

acids have anti-thrombogenic activity. More recently, high prominence is attributed to the role developed by the MUFA, and particularly by the oleic acids that, reducing the oxidation of the cholesterol LDL, may slow the progression of atherosclerosis (Parthasarathy et al., 1990).

As was pointed out above, the P/S (poliunsaturated/saturated) ratio is not suitable measure of the atherogenicity or thrombogenicity of a diet or of foods. Currently they are expressed as follows (Ulbricht and Southgate, 1991):

$$\text{Index of atherogenicity (AI)} = \frac{C12:0 + (4 \text{ x } C14:0) + C16:0}{\text{n - 6 PUFA} + \text{n - 3 PUFA} + \text{MUFA}}$$

$$\text{Index of thrombogenicity: (TI)} = \frac{C14:0 + C16:0 + C18:0}{(0.5 \text{ x MUFA}) + (0.5 \text{ x n - 6 PUFA}) + (3 \text{ x n - 3 PUFA}) + (\text{n - 3 PUFA/n - 6 PUFA})}$$

From how above, Infascelli et al. (2003) carried out a trial aimed to deep the knowledge on the cholesterol content and on the fatty acids profile of buffalo meat. Eight male buffalo calves, after the weaning (63 days) got using an acified milk replacer, received *ad libitum* hay and a maximum of 2 kg/head/d of concentrate; corn silage was administered *ad libitum* starting from 120 d. Reached 6 months of age, the calves were fed, in the amount of 100 g DM/kg of metabolic weight, a diet based on corn silage, rye-grass hay and concentrate (VFU/kg DM 0.84; 14.1 % CP). All the subjects were slaughtered when reached around 400 kg of live weight. The cholesterol content (48.8 ± 2.9 mg/100 g) resulted lower than those found for the Italian bovine bred specialised for meat production (Cutrignelli et al., 2001; Poli et al., 1996). Other authors (Sinclar, 1956) that investigated on the cholesterol content of buffalo meat got values superimposable (46 mg/100 g) or even lower (32 mg/100 g) (Yadava and Singh, 1974). Finally, Cutrignelli et al. (1996), found a not uniform distribution of the cholesterol content. These authors, in fact, reported very different values in groups of buffalo young bulls bred in the same manner: 32.2 ±s 2.5 and 50.6 ±s 2.8 mg/100s g. The contents of myristic, palmitic and stearic acids result slightly different compared to those found by Cutrignelli et al. (1996) for buffalo, but significantly lower than those recorded by Poli

et al. (1996) for young bulls of Chianina bred. To the light of the both high atherogenic and thrombogenic activity of the first two fatty acids and of that only thrombogenic of the stearic acid, Infascelli et al. (2003) attributed a favourable judgment to buffalo meat from the dietetic-nutritional point of view. In fact, although the contents of oleic acid and of PUFA of the ω-6 and ω-3 series were are not particularly high, both the atherogenicity (AI) and the thrombogenicity (TI) index were very low (0.53 and 1.48, respectively). Finally, the authors underlined that the results of the trial might be improved, modifying the breeding technique and mainly the feeding of the animals. In fact, according to some authors (Yadava and Singh, 1974) the use of the pasture would determine an increase of PUFA of the ω-3 series (with high anti-thrombogenic activity in the meat), as the inclusion in the diet, of certain feeds (linseed) can do (Mandell et al., 1997; Scollan et al., 1997).

More recently, Infascelli et al (2005) carried out a research aimed to compare the chemical characteristics, cholesterol contents and fatty acids profile of buffalo meat to those of Marchigiana meat, whose high nutritional qualities are well recognised. For the trial, the right side of 8 buffalo young bulls (fed a TMR with 14.1% CP and 0.84 UFV/kg DM) and of 18 Marchigiana young bulls (fed diet with 15% CP and 0.91 UFV/kg DM), slaughtered at 400 kg and 620 kg live weight, respectively, has been used. The protein content of *Longissimus dorsi* was superimposable (table 16) while buffaloes showed lower fat percentage (1.36 ± 0.1% vs. 2.40 ± 0.6). Concerning the cholesterol, buffaloes showed lower levels (46.4 ± 2.9 vs. 53.7 ± 7.6 mg/100 g) while the fatty acid profile (table 17) was highly different between the species. The buffaloes showed lower percentage of SFA (38.4 vs. 46.6) but higher of MUFA (37.3 vs. 31.16) and PUFA (24.3 vs. 22.3). Thus, due to the different meaning of these fatty acids categories in the equations to calculate the AI and the TI, both were more favourable for buffalo meat (AI: 0.57 ± 0.06 vs. 0.41 ± 0.04 and TI: 1.63 ± 0.13 vs. 1.16 ± 0.13, for Marchigiana and buffalo young bulls, respectively). It must be underlined that the results were surely affected by the different live weight and by the diet fed by animals. However, it is generally suggested to slaughter buffaloes and Marchigiana young bulls, when they reached 400 and around 600 kg live weight, respectively. In addition, the diet fed by Marchigiana young bulls in this trial is largely used while for buffaloes further studies need to individuate the best feeling plane to enhance the dietetic-nutritional characteristics of meat.

1.2.5. *Buffalo meat and human health*

The function of meat in human nutrition has been the focus of intense debate in the last decades. Common wisdom, several pathophysiologic data, and observational studies provide an apparently strong mechanistic link between increased meat consumption and adverse health and economic effects (Keys, 1980; Barnard et al., 1995; Brunner et al., 2008; Trichopoulou et al., 2009), including higher prevalence of adverse cardiovascular risk factors and ensuing atherosclerotic disease. However, given our evolution as hunter gatherers used to a meat-based, yet non-atherogenic, diet (Cordain et al., 2002), it can be easily explained that moderate amounts of selected meat types and cuts are remarkably safe and may even improve the serum lipid profile (Watts et al., 1988). Accordingly, several authorities recommend a return to a diet and lifestyle more in line with our Palaeolithic genome, thus becoming a twenty-first century hunter gatherer, avidly consuming low-fat meat (O'Keefe and Cordain, 2004). These conclusions are also shared by a comprehensive systematic review appraising the evidence in support of a causal link between dietary factors and coronary heart disease, which poignantly concludes that insufficient evidence of association with coronary heart disease is currently present for meat, eggs, and milk. Indeed, not all types and cuts of meats are born equal, as huge variations in composition of meat are well established, and this heterogeneity might by itself explain such uncertainty (United Nations Food and Agriculture Organization, 2000).

Despite such favourable composition of water buffalo meat in terms of cardiovascular profile, no study has so far appraised the impact of consuming buffalo meat on cardiovascular risk or events. Giordano et al. (2010), in a cross-sectional study, demonstrate that water buffalo meat could confer significant benefits in terms of cardiovascular risk profile (Figure 7); they compare recent consumers of buffalo meat vs. subjects who had never consumed buffalo meat vs. long-standing consumers of water buffalo meat.

Several important cardiovascular risk features were appraised at baseline and at 12-month follow-up in 300 adult subjects divided in three groups homogeneous for age, gender, height, body weight, and the remaining diet. At the end of the study, recent consumers of water buffalo meat showed a significant decrease in total cholesterol and triglycerides levels, lower pulse wave velocity, as well as a more blunted response to oxidative stress from baseline to follow up in comparison with subjects who had never

consumed water buffalo meat. The authors concluded that consumption of meat of the water buffalo seems associated with several beneficial effects on cardiovascular risk profile, including lower carotid atherosclerotic burden and susceptibility to oxidative stress. Awaiting further randomized clinical trials, this study suggests that a larger consumption of buffalo meat could confer significant cardiovascular benefits, while continuing to provide a substantial proportion of the recommended daily allowance of protein.

1.2.6. *Organoleptic characteristics of buffalo meat*

Sensory or organoleptic quality remains another important aspect that define the meat preferences. The experience of the market in other countries (Judge et al., 1989) shows that the quality perceived by the majority of end-customers, refers much to the sensory character of the beef. Thus, different organoleptic attributes perceived as a whole, govern the acceptability of the product by the consumer and his willingness to pay a good price for repeat the experience.

Among the attributes that influence the satisfaction, stressed the tender (tenderness), juiciness and flavour of cooked meat (Judge et al., 1989). Of these three factors tenderness plays the most decisive role (Shackelford et al., 1995ab, 1997ab). The other sensations, especially the juiciness and the amount of connective tissue (chewing waste) are closely related to the tenderness assessed by tasters (Jerez-Timaure et al., 1994, Huerta-Leidenz et al., 1997).

Colour

The colour of the meat is usually the first criterion of choice of consumers when buying, as this feature is often associated with the freshness and tenderness. The colour is mainly due to the pigment content, particularly in the myoglobin formed by a nucleus of haematin, consisting of four pyrrole rings linked to a central atom of bivalent iron and a globin. The colour is determined by both the concentration of myoglobin, either by its degree of oxidation. The bright red colour of meat is due to which we are accustomed, in fact, to the oxyhemoglobin that is formed on the surface because the oxygen binds to iron. The colour, therefore, is more or less intense in relation to the amount of myoglobin present, which in turn varies according to species, sex, age of the animal and

the type of muscle. After long periods of exposure the pigment forms methaemoglobin (Fe trivalent), brown in colour, which alters the colour of the meat if it represents 60 % of the total pigment (Brooks, 1938).

The colour of the meat must be evaluated not only in terms of intensity, but also for its stability during storage. These parameters depend on the final pH, the brightness and structure of muscle tissue which affects the absorption and diffusion of the incident light. Immediately after slaughter, the meat appears dark and translucent because the incident light is low.

At pH 5.5, the muscle becomes more opaque, and then emits a larger amount of light that seems clearest. At refrigeration temperature, myoglobin is oxidized to oxyhemoglobin bright red. If the final pH is greater than or close to 6, colour of meat is dark, similar to that of muscle pre-rigor mortis. At high pH water is strongly linked to the protein structure, the muscle has a "closed" structure and the spread of the incident light is low, cellular respiration is also significant and oxyhemoglobin, which is formed on the surface, is continuously transformed into reduced myoglobin, that has a dark red colour. When, however, the pH of the meat drops below 5.9, there is a denaturation of muscle proteins, including myoglobin, resulting in a light coloured meat, which is more opaque, has an open and diffuses better the incident light (Parigi-Bini and Someda, 1989).

The colour is a characteristic easily detectable, although it is too vague, with the simple visual inspection. However this parameter as well as being very subjective, is strongly influenced by illumination, in particular the quality and intensity of light as well as the background colour. Iacurto et al. (2002) describing the buffalo meat color, reported that the behaviour of colour in buffalo is similar to beef but, at least for the slaughter age differences seem more pronounced, because the age affects all three colours parameters: brightness and the dye decreases (-19.7% and -21.5%), the chrome increases (+20.9%), while in cattle increases the brightness and hue does not seem so different in weight classes. The muscle *Longissumus dorsis* (LD), also on the buffalo, has the colours parameters in the middle level except for the chroma where they finds the lowest value (-11.1% vs. *Semimembranosus*), thus confirming LD like the muscle reference. The brightness decreases very rapidly with increasing age: in animals slaughtered at 18 months, the brightness is between -19.7% and -16,0% compared to animals slaughtered at 6 months. The chroma increases with age, particularly among the animals slaughtered

at 6 and10 months and between 14 and 18 months an average increase of 10.0% for all muscles was observed. The tint decrease with age, mainly between 6-14 months (-11.0% from 6-10 month and -7.0% between 10-14 month). The effect of age on the parameters of colour seems to be greater than the type of feed intake and differences between muscles were significant in all age groups.

About these parameter, Infascelli et al. (2004) found that the colour decrease with age so the young buffalo bulls show a darker coloured meat, with a hue closer to magenta, but with less intensity.

Flavour and scent

Many of the psychological and physiological reactions that awakens the meat derived from its flavour and scent. The sensations linked to the taste and aroma results from a combination of factors and are described by Judge et al. (1989). Taste perception involves four basic sensations (salty, sweet, acid and bitter) by the taste buds of the tongue, the aroma, for its part, is detected by many volatile materials that stimulate the nerve endings in the nasal passages. The overall feeling is a combination of gustatory and olfactory stimuli and it is influenced by factors such as texture, temperature, pH, and cooking method.

Meat components responsible for flavour and aroma have not been fully identified. Various sensations are developed as a result of cooking meat, many constituents of muscle tissue, connective and adipose (fat) become volatile compounds and release of volatiles and aromatic juices. It is thought that most of the aroma comes from fat, but Dransfield et al. (1981) stated that both fat and lean fraction contribute to the "flavour" the fat would be primarily responsible for differences between species, while the lean tissue affect on quantitative differences within the species. The more muscles used in the animal's life have a more pronounced flavour because they have more derivatives of phosphorus compounds that store energy. The flavour and aroma that makes one species different from another, it must be of materials that emerge from the fat to cook the meat (Judge et al., 1989). Currently the consumer requires tasty beef, but not smell and taste too intense. It is recognized that the increasing age of the animal the meat’s flavour is more intense, even in unpleasant subjects that are too old.

Irurueta et al. (2010) describing the effect of three aging periods on quality traits of buffalo meat, found that flavour and odour scores corresponded to slightly intense and

amount of connective tissue to practically nothing, for the all aging periods studied.

Infiltration of fat or marbling

Consumers do not like meat too fat. An adequate degree of marbling is, however, essential to ensure the organoleptic characteristics desired by consumers who, mistakenly, believes that the presence of a slight infiltration of fat corresponds to a much higher fat content than the real one (Dell'Orto and Sgoifo-Rossi, 2000). The presence of fat infiltration, in fact, gives the meat some positive characteristics, such as increased tenderness, juiciness and flavour (Jeremiah, 1998; Sañudo et al., 2000a,b).

Juiciness

The meat juices play an important role in consumer's overall impression of the palatability of the meat (Judge et al., 1989). The juiciness is a qualitative aspect evaluated by the feeling of moisture received during the first acts of mastication (initial juiciness) and the persistence of this feeling, due to the amount of fluid that is released during mastication (prolonged juiciness). These contain many of the components of the flavour and help the softening and fragmentation of the meat during chewing. regardless other attributes of the meat, the absence of juiciness limits the acceptability and destroys their unique sensory virtues. When the lipids combine with the water producted from the cooking a broth formes and is retains in the meat, and then squeezed during chewing. This broth stimulates saliva's production, which gives an impression of sustained juiciness. Juiciness usually refers to the cooked meat and it's greater as lower is the loss of liquid during cooking. Lapitan et al. (2007) report that water holding capacity, tenderness, firmness and marbling score in buffalo beef were all comparable to the cattle beef.

Tenderness

The tenderness of the meat is certainly, after the colour, the most important organoleptic characteristic for the consumer and can be defined as the reduced resistance offered to the process of chewing, or cutting treatments and compression in the case of objective tests. At the time of purchase the consumer evaluates the tender considering the colour and the connective tissue in the transversal section of the meat. The opinion thus

expressed is so subjective even if actually darker meat are usually provided by older animals and are less tender, while the presence of thick connective tissue is synonymous with a coarse texture. The term "texture" refers to the way they are adhering particles of a body or substance and, in the case of meat, the arrangement of different cell types and tissues (Kramer, 1972).

The tenderness is a complex feature because several factors contribute to determine it, the most important are: the meat structure, in particular the length of sarcomeres and muscle fiber size, the consistency of the aponeurosis, the amount of fat, the water content, the quality of connective tissue and the amount of collagen. Barton-Gade et al. (1988) found that the tenderness of the meat is directly related to four main factors:

- the muscle fiber degradation
- the contractile state of muscle
- the amount of connective tissue
- the amount of intramuscular fat or marbling.

These factors are susceptible to genetic or environmental variation. Some estimates (Cundiff, 1992) indicate that the additive or genetic effect controls 30% of the variation in tenderness and 70% can be affected by environmental or non additives factors. The tenderness is inversely proportional to the cut force normally measured with the Warner Bratzler blade (FCWB). The other way to describe the tenderness is with the qualification of taster and consumer panels.

The myofibrillar structure acts negatively on the tenderness, especially if the meat is not mature enough or if the body has been cooled too quickly. This mature refers to the physiological age and not age by the calendar. We evaluate the anatomy of the carcass observing the bone, muscle and adipose tissue. The relationship between chronological age and physiological age would be between races and between individuals of the same race. The immaturity of the animals is usually associated with soft meat (Cross et al., 1984). As animals move into maturity, organizing collagen fibres becomes more complex and therefore less soluble during the cooking, making the cooked meat harder to cut (Cross et al., 1984). During cooking, the action of heat gives rise to the liberation of water and coagulation of myofibril proteins: the two phenomena have led to a surge of cohesion and resistance of the cut. The connective tissue, in particular, collagen, is an element of the structure of the muscle that is directly involved in the definition of the

hardness of the meat by the stiffness properties of the fibres that constitute it. The overall variability of tenderness at a given age of the animal can't be explained only by the content of connective tissue, in fact, the conformation of the collagen molecules, their distribution in the connective tissue within the muscle, play a considerable role in determining the tenderness of a muscle. The connective tissue increases with age, rapidly between 8 and 12 months of life and very slowly in the next, during which, however, increases its mechanical strength due to the progressive increase of cross-links between polypeptide chains of collagen. The aging of collagen involves a radical change of its physical and chemical properties and the collagen becomes, in fact, progressively less soluble.

Some studies suggest that differences in tenderness between different breeds is related to the variation in the activity of the calpastatine (Shackelford et al., 1995b; Wulf et al., 1996) who is an inhibitor of calpain enzyme that in turn is a protease that involved in the process of muscle proteolysis. This enzymatic process has been attributed to calpain, the most responsible for *post-mortem* meat tenderness (Koohmaraie, 1991; Koohmaraie et al., 1992). Tenderness and activity calpastatine are characterised by heritability (Koch et al., 1982).

The meat from adult bulls not castrated is qualified by the tasters as less tender when compared to meat from castrated or heifers of the same age (Huerta-Leidenz and Rios, 1993). The reason for the relatively hard character of the bull meat is not very clear. Cross et al. (1984) attribute this to the greater complexity of the connective tissue and increased testosterone levels in the whole animal. Morgan et al. (1993) differ from this assumption and awarded more pronounced activity in the muscles calpastatine of the bulls.

Among the factors listed, the marbling or intramuscular fat is considered an attribute of the juiciness of the meat and therefore also relates to the tenderness. Fat infiltration seems to improve the tenderness because, during mastication, promotes the separation of the muscle fibres and the sensation of juiciness, as it stimulates salivation. The fat, seen as a state of total fat, also acts on the tender limiting the contraction of muscle myofibrils after rapid cooling of carcasses. Wheeler et al. (1994) worked with castrated bulls evaluating 1337 *Bos taurus* and 330 *Bos indicus* and found that the FCWB decreased as intramuscular fat infiltration increased from "trace" to "small" amounts.

Particularly interesting are the numerous rheological research on the characteristics and other physical and chemical characteristics of buffalo meat, from the Institute of Animal Production (Faculty of Agriculture, Portici, Italy) and the Experimental Institute for Animal Husbandry (Rome).

Study by spectrophotometer were carried out to detect in different muscles, the reflectance, the visual brilliance and the dominant wavelength as index of the colour tonality: for all these parameters meat from buffalo calves showed to have an higher neutral component resulting clear and uniform at the different cuts, compared to the meat from cow calves.

Rheological parameters have been determined using texturometer, a machine simulating human chewing. Results comparing meat from buffalo and Friesian calves, slaughtered at 20, 36, 52 and 64 weeks of age (Matassino et al., 1976; Cosentino et al., 1976; Grasso et al., 1982; Cosentino et al., 1982) evidenced that, in any case, buffalo meat is more tender and, therefore, require less chewing time. The higher tenderness is also attributed to the lower content in hydroxyproline in buffalo meat (in 1 g of lyophilised meat: mg 0.669 vs. 0.907; $P<0.001$). Moreover, buffalo meat shows higher power of retention water, resulting, therefore, more juicy. The taste tests (Borghese et al., 1978) conducted using *Longissimus dorsi* roasted on the plate and *Semimembranosus* baked in an oil bath, reveal any substantial differences in organoleptic characteristics between the meat of the two species. These results agree with Ferrara et al. (1964) in relation to tests carried out in Australia, Malaysia, Venezuela and Trinidad; indeed, buffalo steaks have been judged best.

According to Joksimovic (1979) buffalo meat reminds more tender up to a certain age than cow meat, due to a slower growth of the muscular fibre diameter and a lower consistency of the connective tissue. Cosentino et al. (1982) evidenced that Friesian calve between 6.5 and 8.5 months shows lager muscular fibro cells (in terms of maximum and minimum diameter, area and perimeter) compared to buffalo calve of the same age. Charles and Johnson (1975) consider of similar tender, measured with the cut resistance, the muscle of 16 months buffalo calve and 12 months Angus, Hereford and Friesian calves.

The buffalo performances for meat production i.e. growth, feed efficiency, conversion ratio, dressing percentage, carcass evaluation and composition and meat quality cuts, are very important in economic terms but the priority focus for expanding the buffalo

meat market is meat quality, which means chemical, physical, organoleptical and hygienic characteristics and a good presentation to the consumer.

Many years ago a study was undertaken on meat quality in buffalo males slaughtered at different ages. This trial covered 30 Friesian male calves and 30 Mediterranean Italian buffaloes reared under identical feeding and environmental conditions and slaughtered at 20, 28 and 36 weeks of age (Borghese et al., 1978). The water buffalo meat, upon the visual inspection of the judges, was lighter than the bovine meat and a colorimeter confirmed this fact; it became darker with the increasing age of the animals. Cooking losses also decreased with the age of the animal. The meat tenderness using the Warner Bratzler Shear machine and according to a panel taste decreased as the age increased, as did flavour scores, while juiciness scored better after 36 weeks of age.

Many studies have been undertaken in this field comparing buffaloes to Friesian bovines up to 52 and 64 weeks of age, including analysis of the fatty acid composition of subcutaneous, intermuscular, intramuscular, perivisceral and perinephric lipids at different ages (Borghese et al., 1978) but only a few of these results are reported here covering the meat quality of Italian buffaloes fed with hay and concentrates and slaughtered at 52 weeks of age compared with Friesian bovines reared under the same conditions (Borghese et al., 1996). Muscle pH was approximately 6.2 at slaughter, 5.7 after two hours, and 5.5 after six hours. Only the *longissimus dorsi* in bovines showed a significantly ($P<0.05$) higher value (6.2 after two hours and 5.8 after six hours) than other muscles (*semimembranosus* and *semitendinosus*) and than in buffalo *longissimus dorsi*. After 24 hours, the pH was about the same (5.5-5.6) for all the muscles in both species, with a slight increase (5.7) from the sixth day on. Therefore, after ageing, pH characteristics are practically the same in both species. The trial demonstrated that normally there were no significant differences between species in the nine studied muscles for all the physical parameters, using the Instron machine. The hardness of the raw meat tested by the Warner Bratzler Shear machine was significantly ($P<0.05$) higher in buffalo only in the *iliopsoas* muscle, while in bovine bulls it was higher than in buffaloes only in the *semitendinosus*, while significant differences were found in the force used only in the *caput longum tricipitis brachii*. With the compression test only gumminess in the *supraspinatus* was significantly higher in the bovine. The chewiness, that is the synthesis of physical parameters, shows a tendency to be higher in bovine bulls. This could explain why generally people say that buffalo meat is more tender. The results of the Warner Bratzler Shear tests on cooked meat evidenced that

longissimus dorsi was more tender than in the raw meat, particularly if baked; after baking, both species showed the same values, while buffalo meat appeared more tender after being cooked in boiling oil ($P<0.09$). The cooking losses, when the meat temperature reached 70°C, were about 21 percent after boiling in oil, and about 12 percent after baking, with no differences between species. Further quantity was lost, ten minutes after cooking with liquids: seven percent after cooking in boiling oil, and approximately six percent after baking, with the same trend for both bovine and buffalo meat.

The percentage of judges that identified the species was 22.9 % for meat slices cooked in the open pan and only 7.5 % for meat cooked by pressure cooking, less than casual probability. The percentage was significantly ($P<0.01$) higher for slices cooked in the open pan, where the meat was less cooked and quite natural. A large number of judges declared impossible to identify the meat (52.0 to 74.5 % for the open pan and pressure cooker respectively), while 25.1 and 18.0 % respectively mistook the identification. No judge identified the species in all the tests. No difference was found with regard to the evaluation of tenderness, flavour and juiciness. Only in two taste tests was buffalo meat significantly overscored by one judge on the panel. The judges always gave better scores ($P<0.05$) to the meat cooked in the pressure cooker than that cooked in the open pan (Borghese et al., 1996). Regarding the tests on tenderness values undertaken with the Instron machine on several muscles of Italian buffaloes slaughtered at 190 days, Failla et al. (2001b) found values in raw meat varying from 2.98 to 4.87 kg/cm, while Tonhati et al. (2001) found a mean of 4.52 kg/cm in Murrah 30 months old.

1.3. Aim of the thesis

The general aim of the PhD thesis, realised at the Department of Animal Science and Food Control (University of Napoli, Federico II, Italy), was to study animal performance and the nutritional characteristics of meat from Buffalo bred in Italy (*Italian Mediterranean Buffalo*) fed different diets.

The importance of this research is to give a contribute to better characterize the buffalo *infra vitam* performance (i.e. weight gain, feed conversion index, etc.) and meat quality (mainly in terms of fatty acids profile). Moreover, the originality of the investigation into the field was the diet composition.

In particular, in the first experimental study the use of a leguminosae (faba bean, *Vicia faba minor* L.), as protein source in the diet of buffalo, was compared to the soya bean meal.

The second investigation refers the results obtained using a nutraceutical additive (*Aloe arborescens*) in the dairy cow buffalo diets to study the influence on the colostrums quality and buffalo veal performance in the early period of life.

2. EXPERIMENTAL STUDIES

2.1. Favino as a protein source in diet for buffalo bulls

2.1.1. *Introduction*

Since 2001 the European Commission banned the use of meat and bone meal and its by-products in diets for livestock animals (EC directive 999/2001) in order to assure consumer safety on animal products. Consequently, soybean meal became the most utilised protein source in the intensive livestock systems. Moreover the proteins of this source are low degradable in the rumen and well proportioned to the non structural carbohydrates (NSC). Soybean meal solvent extract (s.e.) is a by-product of oil industry, where soybean seeds are treated with organic solvents (e.g. hexane) and subsequently with high temperature. For this reason soybean meal has been banned in the organic livestock (EC directive 2092/1991; EC directive 834/2007). Even if in Europe the high part of soybean is imported, soybean s.e. represents a less expensive protein source for its high crude protein content (44-50 % as fed) (Cutrignelli et al., 2011). However soybean meal costs and availability are strongly related with the price development of agricultural commodities on the world market (Jezierny et al., 2010). Factors which may influence world market prices include variations in population and economic growth, changes in consumer's product preferences, but world market prices are also dependent on weather conditions (Gill, 1997; Trostle, 2008).

Thus, the search for alternative protein sources has led to an increasing interest in the use of grain legumes, as they supply the important source of plant protein. The botanical family of grain legumes is known as *Fabaceae*, also referred to as *Leguminosae*. Grain legumes are cultivated primarily for their seeds which are harvested at maturity, and which are rich in protein and energy. The mature dry seeds of grain legumes are used either as animal feed ingredient or for human consumption (Singh et al., 2007). Beans,

lentils and chickpeas are utilised exclusively for human nutrition, while the other grains are used in animal feeding too. In Italy grain legumes cultivation is progressively increased due the presence of new cultivars more hardy and productive. These new cultivars were selected principally in France and are characterised by lower water requirements, higher production and higher resistance to the parasitic infestations and to the adverse environmental conditions. Generally, legumes are characterised by their ability to use atmospheric nitrogen as a nutrient due to the symbiosis with nitrogen-fixing bacteria from the *Rhizobium* species (Sprent and Thomas, 1984; Zahran, 1999).

Therefore, unlike other cultivated plants, legume crops need less nitrogen fertiliser for optimal growth, and the use of legumes in crop rotation systems reduces the need of nitrogen fertiliser in subsequent crops (López-Bellido et al., 2005). Nitrogen benefits in legume-cereal rotation systems have been attributed not only to the transfer of biologically fixed nitrogen (Díaz-Ambrona and Mínguez, 2001; Evans et al., 2001), but also to lower immobilisation of nitrate in the soil during the decomposition of legumes compared to cereal residues (Green and Blackmer, 1995), also termed as the nitrogen-sparing effect.

Thus, nitrogen benefits may result from a combination of legume nitrogen sparing effects and the bacterial nitrogen fixation (Chalk et al., 1993; Herridge et al., 1995). In addition, crop rotation and intercropping with legumes may provide successful strategies for weed suppression (Liebman and Dyck, 1993; Bulson et al., 1997). Weed growth and development may be disrupted due to varying cultivation conditions prevailing for the different crops used (e.g. fertiliser requirements, planting or maturation dates), thereby preventing domination of only a few weed species (Froud-Williams, 1988; Liebman and Janke, 1990). Due to these pioneer crop effects, cultivation of grain legumes is an important part of crop rotation, particularly in organic farming (Badgley et al., 2007).

In animal nutrition, grain legumes are mainly used as protein supplements, but also as a valuable energy source, due to their partly high contents of starch (faba bean, peas) and lipids (lupins) (Gatel, 1994; Bach Knudsen, 1997; Salgado et al., 2002). However, the use of grain legumes in animal nutrition has been hampered due to partially high concentrations of secondary plant metabolites, also referred as antinutritional factors, including condensed tannins, protease inhibitors, alkaloids, lectins, pyrimidine glycosides and saponins. Possible negative effects of these secondary plant metabolites

include, for example, feed refusals (tannins, alkaloids), reduced nutrient digestibilities (tannins, protease inhibitors, lectins) or even toxic effects (alkaloids) (Rubio and Brenes, 1995; Lallès and Jansman, 1998; Huisman and Tolman, 2001).

However, the ban on use the soy which comes from genetically modified crops in organic farming (Reg. CE 1804/99), has greatly stimulated research on GM-free feeds, which are able to satisfy protein requirements in animal nutrition, and renewed the interest in low input grain legumes, especially for eco-sustainable crop-livestock systems. Moreover grain legumes are strategically important not only in decreasing the marked deficit of high-protein feedstuff (Annicchiarico and Carroni, 2009), but also in increasing the sustainability of crop-livestock systems through the safeguarding of soil fertility, the reduction of greenhouse gas emission, and the reduction of nitrogen fertilizers use, etc. (Carrouée et al., 2003; Jensen and Hauggaard-Nilesen, 2003). Last but not least, the increase of domestic production of GMO-free plant proteins represents a way to reduce Italian dependence on feedstuff imports. Among legumes, lupin appears to be an interesting and promising crop. Since it has a winter cycle, it may play a key role in crop rotation (Postiglione et al., 1995) especially as it has a high protein content compared to the other winter legumes (Hill, 1977; Sujak et al., 2006). However, since the late 1960s their importance has been reduced due mainly to the use of oleaginous meals, mostly soybean which have been available at competitive prices (Gresta et al., 2010). As a consequence, an important reduction in legume grain cultivation was noted (Cubero, 1983). This was due to a lack of genetic and technological improvement. This seems to have happened also in other countries in South Europe (Duffus and Duffus, 1991). In order to reduce the dependence of Europe in imported legume grains as protein sources for animals, the EU has initiated some production incentive policies (Todorov, 1988; Chominot, 1992).

Within the few researchers who have studied the nutritive value of Mediterranean legume grains, special reference must be made to the work of Wiseman and Cole (1988) with pigs and Castanon and Perez-Lanzac (1990) with layer hens, and of Hadjipanayiotou et al. (1985) with sheep, pointed to the possibility of using these grains in animal diets, although in limited amounts. These characteristics increased its use in ruminant diet, since lupin can replace soybean meal even for high-producing lactating cows (van Barnelveld, 1999; Froidmont and Bartiaux-Thill, 2004).

Apart from soybean and lupin species, legume seeds have a high protein (20-30%) and high carbohydrate (50-65%) content. In the high carbohydrate legume seeds, 35-45% is made up of starch, the major energy source for (most) farm animals. However, legume seeds contain a considerable quantity of protein that is highly soluble and rapidly degradable (up to 75%) in the rumen (Arash Azarfar, 2007) which are generally associated with nitrogen loss as ammonia and makes them less suitable as a protein source. To avoid this problem dairy diets are usually supplemented either by a source of highly fermentable carbohydrates or with a source of rumen undegradable protein. The latter can be included in the animal's diet via an ingredient source of undegradable protein, either by nature, or resulting from technological processing. In the feed industry, many forms of technological processing are applied to manipulate the site and extent of digestion in ruminants. Expander treatment is such a process which involves heat, pressure and shear.

Aim of the investigation was to compare a leguminosae (faba bean, *Vicia faba minor* L.), as protein source in the diet of buffalo, with the soya bean meal. Faba bean is widely used in the Mediterranean region as source of protein in both human and animal nutrition (Larralde, 1982). However, the occurrence of some antinutritional factors such as phytohemagglutinins, protease inhibitors, polyphenols, saponins, phytates, etc., has hampered a wider nutritional utilization of this legume (Liener, 1980). The nutritional value of field bean has been traditionally attributed to it high protein content, which ranges from 25 to 35%, despite the imbalance in sulphur aminoacids. Most of these proteins are globulins (60%), albumins (20%), glutelins (15%) and prolamins. It is also a good source of sugars, minerals and vitamins.

Thus, the chemical analysis of this legume reveals a 50- 60% content of carbohydrate, which is mainly constituted by starch, while the proportion of lipids is relatively low at about l-2.5% with oleic and linoleic acids representing about 75% of fats (Matam and Salido, 1985). The mineral content varies between 1-3.5%, being particularly rich in calcium and iron. Additionally thiamin, tocopherols, niacin and folic acid are constituents that can be destroyed by heating, eliminated by soaking or removed by physico-chemical treatments or plant breeding .

2.1.2. *Material and methods*

Animals and dietary treatments

The trial was carried out in 2009 on agro-zootechnical farm situated at 47 m a.s.l. in Southern Italy (Sant'Angelo in Theodice, Cassino, FR), where about 200 Italian Mediterranean buffaloes are bred.

Sixteen calves (about 30 d of age, average weight 55 kg), equally divided into two groups received 6 l/head/d of acidified milk replacer (in the ratio of 180 g/l of water) until 56 d. Subsequently, the replacer amount was gradually decreased, whilst administering the same volume. Roughly chopped alfalfa hay and weaning concentrate were available from the fifth week; corn silage was administered starting from 70 d. After weaning, the animals were fed *ad libitum* hay and corn silage; the concentrate were administered in the amount of 2 kg/d.

After weaning, about 84 d, each animal was placed in individual box up to the slaughtering weight. The animal groups were fed (2.7% body weight, BW) isoprotein (CP: 14.9 % DM) and isoenergetic (0.91 UFV/kg DM) diets, differing in protein source: faba bean (*Vicia faba minor* L.) vs. soya bean meal (*Soja hispida*). The groups were named according to the administered protein source: faba bean (FB) and soya bean meal (SB). As forage a mixed hay and a corn silage produced by the farm were administered to the two groups. The chemical composition and percentage of ingredients of both diets are depicted in table 18.

Every two months, samples of each feed were collected to determine the chemical composition (Van Soest *et al.*, 1991; AOAC, 2000). The nutritive value was calculated as net energy of growth (VFU/kg DM) as suggested by INRA (1988). Individual feed intakes were registered daily to calculate the feed conversion indexes (FCI for dry matter, FCI_DM).

Measurements in vivo and at the slaughter

One day before slaughter, blood samples from each animal were collected at 08.00 h, before feeding, from the jugular vein in vacutainer tubes. The tubes were centrifuged at 3000 g for 15 min. The recovered serum was stored at –18°C until analysis for metabolic profile (Di. LAB. Diagnostica Veterinaria, Napoli, Italy). All the animals were weighed at the beginning of the trial, and thereafter every two months, until BW of

350 kg fixed in advance as slaughter weight, was reached. All animals were slaughtered in an authorized slaughterhouse according to EU legislation (EU Regulation EC No 882/2004). At the last weight control, on the subjects fasting for 24 hours the following measurements were made (ASPA, 1991) as showed in Figure 8: height at withers and at pelvis, round circumference, length of rump, body length, depth of chest, width of pelvis and of chest.

In order to evaluate the carcass characteristics, the following measurements were conducted (ASPA, 1991) on the hot carcasses, as showed in Figure 9: length of carcass, depth of chest, length, width and thickness of leg. Moreover, skin, head, legs, organs and cavitary fat were weighed to calculate hot and cold dressing. "Tare" included blood and all the carcass processing losses. After 15 days of tendering at 4±1°C, the right side of each animal was dissected. Meat, long bones and bones not included in the commercial cuts were weighed in order to calculate their incidences on cold carcasses. The pH in *Longissimus thoracis* (LT), *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles were measured within 1 h from death and at the end of tendering by a Hanna pH-meter (mod. HI 9025) equipped with an electrode FC 230C.

From each right side a sample cut corresponding to the 10^{th} rib was taken (Lanari, 1973). In particular, the dissection was made along the cranial borders of the 10^{th} and 11^{th} ribs (at the dorsal 3^{rd} of the first one) to include the corresponding trait of the 10^{th} rib, half of the body of the 10^{th} vertebra and partially the 11^{th} ones. The spinous processes of the 8^{th}, the 9^{th} and the 10^{th} vertebra were partly taken. In the sample cut part of the following muscles were included: *spinalis, spinalis thoracis, semispinalis thoracis, ileocostalis thoracis, transversospinalis, serratus dorsalis caudalis, latissimus dorsi,* and *longissimus thoracis.* The sample cuts were dissected to calculate the incidence (%) of separable muscles, bones and fat.

Analytical determinations on meat samples

Samples of *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Iliopsoas* plus *Psoas minor* (IP) muscles were collected and rapidly transported, upon refrigeration temperature, to the laboratories for the chemical analysis. The muscles were homogenised, divided into different aliquots, vacuum-packed and frozen (-20°C) until the analysis (chemical composition, cholesterol content, fatty acid composition). For chemical composition (moisture, crude fat, ash, protein and collagen), the analysis were

assessed, after 24 h thawing at 4°C using a food analyser (FoodScan Lab, FOSS Electric, Denmark). For the cholesterol determination, the extraction was made according to Naaemi et al. (1995); for its quantification, as suggested by Indyk (1990), a HPLC equipped with diode-array detector (mod. 996, Waters) and computer integration system Millennium32 was utilised. The operative conditions were the following: temperature 20°C; mobile phase hexane/isopropyl alcohol (99/1); flow 1 ml/min; Phenomenex Bondclone C18 column (250x4.6 mm); loop 10 µl; wave-length 212 nm; external standard Cholesterol (SIGMA C-8667).

For the determination of fatty acids profile, we take advantage of the collaboration with the group of Animal Nutrition of the Faculty of Veterinary Medicine, University of Messina (Italy). They were determined by gas chromatography (GC). To this purpose total fat was previously extracted (Folch et al., 1957) and subsequently turned into methyl esters (FAMEs) by direct transesterification (Christie, 1993). The oil extracted from each sample was suspended in a mixture of sulphuric acid/methanol (1:9, ml/ml) and heated for 3 h. The FAMEs were isolated by adding 1 ml of n-hexane. The mixture was shaken and after 2 min the formed top-layer with n-hexane was transferred into the vial for the GC injection.

The FAMEs were analyzed by GC-FID (Agilent Technologies 6890N, Palo Alto, CA, U.S.A.) with a split/splitless injector, a flame ionization detector and fused silica capillary column Omegawax 250 (Supelco, Bellefonte, PA, U.S.A.), 30m x 0.25mm I.D., 0.25 µm film thickness. Column temperature was programmed: initial isotherm of 160°C (6 min.), increment of 3°C/min and final isotherm of 250°C (30 min.). Temperature of the injector and detector: 250°C. Injection volume: 1.0 µL. Carrier gas: helium (1 mL/min). Split ratio: 1:50. Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from Supelco (Bellefonte, PA, U.S.A.).

Chromatogram peak areas were acquired and calculated by Chemstation software (Agilent, Palo Alto, CA, U.S.A.). The concentration of each fatty acid was expressed as g/100 g, considering 100 g the summation of the areas of all fatty acid methyl esters identified. For each sample the chromatographic analysis was replicated three times.

Data processing

The parameters referred to living animals [body weight, daily weight gains, biological

efficiency of growth (BEG = DWG/$BW^{0.75}$ and FCI] were calculated from the data of the individual curves of growth as suggested by Pilla et al. (1987) and Pilla (1991). In order to evaluate the better equation to describe the curves of growth of young bulls, the following equation were used (PROC REG, SAS, 2000):

quadratic: $y = = a+bx+cx^2$

allometric: $y = a \cdot (x^b)$

Moreover, the values of the classes of fatty acids considered of particular nutritional significance, were obtained from the summation of the values of various fatty acids:

- SFAs, sum of the values of C14, C15iso, C15anteiso, C15, C16, C17iso, C17, C18, C20, C22, C23, C24;
- MUFAs, sum of the values of C14:1cis-9, C16:1-n7, C17:1cis-10, C18:1trans-10, C18:1trans-11, C18:1-n9, C18:1cis-11, C20:1cis-11, C24:1cis-15;
- PUFAs, sum of the values of C18:2-n6, C18:3-n6, C18:3-n3, C20:2cis-11,14, C20:3-n6, C20:3-n3, C20:4-n6, C20:5-n3, C22:4-n6, C22:5-n3, C22:6-n3;
- CLAs, sum of the values of CLA-cis-9-trans-11 and CLA-trans-10-trans-12.

In order to understand how the buffalo meat can reduce the risk of Cardiac Health Disease (CHD) development, the Atherogenic Index (AI) and the Thrombogenic Index (TI) were calculated as follows, according to the suggestion of Ulbricht and Southgate (1991):

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{n\text{-}6\ PUFA + n\text{-}3\ PUFA + MUFA}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times MUFA) + (0.5 \times n\text{-}6\ PUFA) + (3 \times n\text{-}3\ PUFA) + (n\text{-}3\ PUFA/n\text{-}6\ PUFA)}$$

Statistical analysis

Live, slaughtering and dissection data were statistically processed to determine the differences between the protein sources (faba bean vs. soya bean meal) using the SAS (2000) GLM procedure, according to the following model:

$$y_{ij} = \mu + PS_i + \varepsilon_{ij}$$

where: y = dependent variable, μ =mean, PS= protein sources effect (I = FB, SB) and ε = error.

For the meat quality parameters (chemical composition, cholesterol and fatty acids profile), the statistical analysis was performed with the following model:

$$y_{ijk} = \mu + D_i + M_j + (D*M)_{ij} + \varepsilon_{ijk}$$

where: y = dependent variable, μ = mean; D = dietary effect (i = FB, SB), M = muscle effect (j = LT, ST, IP), D*M = interaction diet x muscle and ε = error. The mean were statistically compared using t-test ($P < 0.05$).

2.1.3. *Results and discussion*

The metabolic profile of the young bulls (about 420 d of age, average weight 346 kg) of the two groups measured at the end of the trial is depicted (table 19). No difference were between the two groups. Most of the values obtained fall in the range reported in the literature (Kaneko et al., 1997; Duncan et al., 1994; Borghese et al., 2010) and considered as reference values for adult buffalo (mg/dl: glucose 33-66, urea 8-40, creatinine 0.90-1.75, UI/l: GOT 43-127 and GPT 14-35). However, the parameters of the energetic metabolism were lower than those reported by others (cholesterol: 42.7 vs. 65-120 mg/dl and triglycerides 11.5-15.6 vs. 33-45 mg/dl). De Palo et al. (2005), in a study with water buffalo calves with more than 101 days of age, reported higher value for all these parameters: glucose (67.45 mg/dl), urea (39.92 mg/dl), creatinine (1.36 mg/dl), cholesterol (78.8 mg/dl), triglycerides (23.4 mg/dl), GOT (190 UI/l) and GPT (57 UI/l).

Infra-vitam performance and post mortem measurements

Table 20 shows the average parameters of individual growth curves for each group obtained by measurements at different ages. In order to obtain the individual growth curves, the quadratic function (weight = $a + b \cdot age + c \cdot age^2$) was chosen because this model gave higher determination coefficients than the allometric function in accordance with Di Lella et al. (1998) and Cutrignelli et al. (2008a). As expected, body weight is strictly correlated to the age of the animals. The results demonstrate that the young bulls

until 420 days of age have high daily weight gain and not show yet a decrease, typical of the terminal phase of body development. From the equation results that the intercept with the ordinate, so the estimated BW at birth, is 33.66 kg in FB group and 36.47 kg in SB group.

Table 21 and Figure 10 show the *infra-vitam* performance of the buffalo young bulls of the two groups. All the animals show good daily gains, almost 800 g/d, showing an acceptable growth capacity until 420 kg of live weight, similar to that found in Italian Mediterranean Buffalo by Borghese et al. (2010) .

Only few differences (not statistically significant) appear between the two group, in particular BW was superimposable until 330 day of age and then slightly decrease in FB group. Similarly, the registered DWG was higher in SB group after 180 day of age and, on average, for all the investigation period (DWG 60-420 d: 0.768 vs. 0.797 kg, for FB vs. SB respectively), but the differences were not significant, indicating that the replacement of soya bean meal s.e. with faba bean seeds did not affected the regular growth of the buffalo bulls, mainly in the first period of growth. The differences are probably due to the higher non-protein nitrogen (NPN) concentration of the faba bean than the soya bean meal (about 12 vs. 1.3 % of CP, respectively, according to Bovera et al., 2001) and the anti-nutritional factors of faba beans, such as proteinase inhibitors, phytic acid and tannins (McDonald et al., 2002).

Concerning the influence of a different protein source on young bulls growth, Moss et al. (1997) found no significant effects on weight gain and feed intake when soya bean meal was replaced on an isonitrogenous basis by lupin seeds in diets for growing cow bulls (BW from 182 to 243 kg); similar results are reported by Kwak and Kim (2001) on Korean native bulls (BW from 247 to 427 kg) utilising two different concentrations (15 and 30%) of flaked lupin. Instead, our results agree with our previous results obtained on Marchigiana young bulls (Cutrignelli et al., 2008a) where the protein source (soya f.e. and faba bean) did not affect any *infra-vitam* parameters except the BW at 180 d of age (173 vs. 186 kg, soya and faba, respectively; $P<0.01$).

Concerning the body measurements carried out *in vitam* and at the slaughter (table 22) any difference were observed between the two groups, excepted for the body length, which values resulted higher in FB compared to SB group (117 vs. 82 cm, respectively; $P<0.01$). Both groups show higher height at pelvis compared to the height at withers (123 vs. 120 cm) and a development of the body length (100 cm) lower compared to the

height at withers and pelvis. Moreover, the index body-chest [= (body lenght * 100)/round circumference], indicating a strong “dolicomorfismo” if its value, is close to 100%, is on average 59.6%.

These results show that the Mediterranean buffalo slaughtered at about 400 days of age appears to be still in a stage of full morphological development, showing a satisfactory increases in daily weight gain, associated with a greater development in body width and length than the height measurements. As reported by De Palo et al. (2005), the growth of the young buffalo bulls is typically bradiauxesico type, because the shape of the body show a long trunk and tall, underdeveloped limbs, poor air in the stomach and transverse diameters of good developments. These results can be encouraging for the use of buffalo bulls for meat production.

Analysing data from the dissection, reported in table 23, results that protein source influenced neither body and carcasses conformations nor dressing out, confirming that faba beans could be used as an alternative protein source to soya bean meal s.e. in the intensive livestock of meat bulls. In addition, the dressing out at slaughter showed values quite high (net hot dressing out: 53.8 %), even if lower than other obtained in previous study (about 60%; Infascelli et al., 2001). These results, are very interesting considering that any genetic selection has been made until now in the Italian Mediterranean buffalo finalised to the improvement of the meat production.

The results of the sample cut dissection (table 23) indicate any significant difference between groups FB and SB; however, the meat incidence was higher and the fat percentage lower in SB group compared to FB group (fat: 20.07 vs. 22.90 %; meat: 55.55 vs. 52.46 %, for SB and FB, respectively). However, it is important to underline that the sample cut measurements is often contradictory and conflicted with the data obtained from total carcass dissection (Cutrignelli et al., 2008a).

The pH values of the tested muscles (*Longissimus thoracis*, *Semitendinosus* and *Semimembranosus*) indicated a correct process of acidification (Figure 11), as indicated by Warriss (2000): the slaughter values were always lower than 7.0 and the dissection values always higher than 5.5. No significant differences were found as function of the feeding schemes for each tested muscles.

Meat quality

In Table 24 the chemical composition of the three tested muscles (*Iliopsoas* plus *Psoas minor, Semitendinosus, Longissimus thoracis*) of both groups is reported. As a whole, the chemical composition confirmed the data reported by Cutrignelli et al. (1996), however fat and collagen content in the present study were particularly low (average values: 1.68 and 1.90 %, for collagen and fat, respectively). The cholesterol content we have found (33.3 %), although sensibly higher than that reported by Samoggia et al. (1993) in the gracilis muscle (mg 27.7) and the diaphram (mg 31.4) is nevertheless lower than that obtained by Cutrignelli et al. (1996) in young buffalo bulls *Longissimus dorsi*. The contents of cholesterol in buffalo meat in the literature are rather disomogeneous: Kesava et al. (1992) found 83 mg/l00 g in the *Longissimus dorsi*; Yadava and Sigh (1974) indicated a sensibly lower content (mg 32); Sinclair et al, (1982) found 46 mg in Australian buffalo meat.

The type of muscle highly ($P<0.001$) affected every parameters, excepted collagen ($P<0.05$) and cholesterol ($P>0.05$); in particular, the ST samples, which correspond to the silverside, showed in both groups (figure 12) significantly lower fat ($P<0.01$) and higher protein ($P<0.05$) concentrations, but higher cholesterol content ($P>0.05$) than the other two muscles. Several authors found no differences in cholesterol contents among muscles (Bohac and Rhee, 1988; Cifuni et al. 2004), while others (Eichhorn et al., 1986; Browning et al., 1990; Rusman et al., 2003) found significant differences. This contradiction is probably due to the different muscles analysed in each experiment: indeed Bohac and Rhee (1988) and Cifuni et al. (2004) utilised the *Longissimus thoracis*, the *Semimembranous* and the *Semitendinosus*, while Eichhorn et al. (1986) and Browning et al. (1990) analysed the *Biceps femoris* and *Longissimus thoracis*. As theorised by Wheeler et al. (1987) the cholesterol content may be affected by the different physiological function of the muscles.

As evidenced in table 24, the different protein only slightly ($P<0.05$) affected the fat, protein, collagen and cholesterol content; in particular, FB group showed lower fat and cholesterol, but higher collagen and lower protein compared to SB group.

Tables 25, 26 and figure 13 report the fatty acid profile (g/100 g) and the atherogenic (AI) and thrombogenic (TI) indexes respectively of IP, ST and LT and FB and SB groups. Most of acids are significantly ($P<0.01$) affected by muscle type, excepted C18:1-trans10 and C18:1 trans11, trans10-trans11 CLA, C24:1-cis15 and C22:6n-3. On the other hand, protein source significantly influenced only C17:1-cis10, C20, C22,

C20:3n-6, C20:4n-6, C24, C24:1-cis15, C22:5n-3, C22:6n-3 ($P<0.05$) and C15-anteiso, C15, C17, C23 ($P<0.01$). According to the observations of some authors (Morris et al., 1995, Cifuni et al., 2004, Migdal et al., 2004) palmitic (C16:0), stearic (C18:0) and oleic (C18:1-n9) acids are the most widely represented fatty acids and, are not statistically different between the two groups.

Concerning some fatty acids considered of particular nutritional significance (table 26), the protein source affected only SFA which were significantly ($P<0.05$) higher in SB group (50.3 vs. 49.9) and PUFA that were slightly ($P>0.05$) higher in FB group (13.4 vs. 12.8).

Different results are reported by Cutrignelli et al. (2008b) that found significantly ($P<0.01$) higher value for stearic acid in bulls fed faba bean than in those fed soybean meal s.e.; however this result did not significantly affect the SFA concentration, or AI and TI indexes.

Compared to the data reported by Cutrignelli et al. (2008b) on buffalo young bulls, our SFA and UFA were, respectively, slightly higher (50.1 vs. 43.7) and lower (49.6 vs. 55.3). In our study, also the ratios between such acids (PUFA/SFA, ω-6/ω-3, AI, TI) were never different between the two groups.

The AI of the buffalo meat in this trial was particularly interesting, lower than the data reported by Ulbricht and Southgate (1991) for raw minced beef and than those reported by Badiani et al. (2002) for cooked beef (0.72 and 0.77, respectively); our data were similar to those reported by Cutrignelli et al. (2008b) on Marchigiana young bulls (0.52). However, TI in this trial was similar than the findings of the above-cited authors (1.27, 1.30 and 1.33 for Ulbricht and Southgate, 1991, Poli et al., 1996, and Cutrignelli et al., 2008b, respectively) and lower if compared to TI (1.77) reported by Badiani et al. (2002).

Differences highly ($P<0.01$) significant were found comparing the three muscles analysed, and, also in this case, ST showed the most favourable fatty acids profile: lower SFA, higher PUFA, MUFA, ω-3, ω-6, CLAs and, consequently, lower values for both index (AI: 0.60, 0.42, 0.57 and TI: 1.50, 1.02, 1.51, for IP, ST and LT, respectively). Several studies have demonstrated that PUFA have a positive impact on human health; the beneficial effects of n−3 fatty acids have been shown in the prevention of coronary heart disease, hypertension, type 2 diabetes, rheumatoid arthritis and some other diseases (Simopoulos, 1999). Especially conjugated linoleic acid (CLA)

is suggested to have immunomodulating, anticarcinogenic and antiartheriosclerosis properties (Pastuschenko et al., 2000; Whigham et al., 2000). CLA is a group of positional and geometric fatty acid isomers derived fromlinoleic acid of which milk fat is the richest dietary source (Parodi, 1999).

Muscle with a high percentage of unsaturated fatty acids (UFA) generally scored higher in taste panel evaluation (Westerling and Hedrick, 1979) and food with high UFA, especially PUFA, is good for human health (Rusman et al., 2003). The ω-6/ω-3 ratio was higher than the value (less than 3) reported by Scollan et al. (2006) but lower than that registered by Warren et al. (2003) for steers fed corn silage and concentrates (8.9).

The interactions between muscle and diet for the fatty acids profile was always not statistically significant ($P>0.05$) and in most of the case (figure 13) for each parameter in the three muscles follow the same trend in both groups.

2.1.4. *Conclusions*

The results show that the faba bean could be used as an alternative protein source to soya bean meal s.e. as it did not affect the growth rate (body weight, daily weight gain and biological efficiency of growth) or the feed conversion indexes during the whole experimental period, and offers decided agronomical, economical and healthy advantages. Moreover, replacing soybean meal s.e. with faba bean in the diet for young bulls does not substantially influence the nutritional characteristics of meat.

Although the meat of group fed faba bean had significantly higher concentrations of some fatty acids compared to the level found in SB group, neither the atherogenic and thrombogenic indexes, nor the cholesterol content were influenced.

From these results it is possible confirm the favourable assessment of the nutritional characteristics of the meat from buffalo young bulls. Moreover, the fatty acids profile of muscle confirms that the meat of the buffalo, even if not specialised in meat production, has higher unsaturated fatty acids concentration and lower saturated fatty acids levels, which in turn ensures low atherogenic and thrombogenic indexes.

2.2. Aloe supplementation in pregnant buffalo cows to improve colostrum quality

2.2.1. *Introduction*

Buffalo calves depend on the passive transfer of colostral IgG to provide humoral immunity during the neonatal period (Barrington and Parish, 2002) and adequate passive transfer of immunity, determined by measurement of serum IgG concentration, is a critical determinant of short-term health and survival for neonatal calves (Constant et al., 2004). To ensure adequate passive transfer of immunity, neonatal buffalos should receive good quality colostrum in an amount equivalent to 5% of their body weight divided into 4 to 6 feedings of equal proportions, preferably within 3 to 12 hours after birth. Failure to ingest and/or absorb sufficient colostral IgG, termed FPT, is a secondary immunodeficiency condition that has been linked to increased risk of illness and death from bacterial septicemia and common neonatal infectious diseases and is well recognized among ruminant species. Calves with FPT have an increased risk of illness and death until at least 6 to 7 weeks of age. The influence of management practices on passive transfer of immunity is well established for several ruminants. Administration of colostrum substitutes has had limited success in terms of calf survival rate. Increases in neonatal morbidity and mortality rates are well accepted consequences of FPT among juvenile ruminants, both before and after weaning (Argüello et al., 2004). Nevertheless, relatively little has been done in the recent years to identify possible feeding supplies that may be able to improve passive immunity in calves and, additional, very little has been done concerning buffalo, despite newborn mortality has been reported to be very high in such species. In such contest, the genus Aloe plant, whose four species namely, *Aloe barbadensis Miller* (syn. *Aloe vera*; Liliaceae), *Aloe ferox* (syn. *Cape Aloe*; Liliaceae), *Aloe arborescens* (syn. *Candelabra Aloe*; Liliaceae) and *Aloe perryi baker* (syn. *Perry's Alo*; Liliaceae) have been traditionally applied for the medicinal practice over thousands of years in many cultures of the world, could play an interesting rule. Indeed, the polysaccharide fractions of Aloe have been reported as potent B cell stimulators either *in vitro* (Leung et al., 2004) and *in vivo* (Liu et al., 2006)

studies.

Aloe is native to southern and eastern Africa, and subsequently introduced into northern Africa, the Arabian peninsula, China, Gibraltar, the Mediterranean countries and the West Indies. It is commercially cultivated in Aruba, Bonaire, Haiti, India, South Africa, the United States of America, Central America and Venezuela. *Aloe* has been called the "plant of immortality" because it can live and bloom without soil. It was given as an offering at the funerals of pharaohs and used in the baths of Egyptian queens. Today, Egyptians still hang an aloe plant over the door of a new house to provide a long and fruitful life for its occupants. According to the Roman scholar, Pliny, the plant was also used for embalming. The Greek physician Dioscorides used aloe gel for mouth infections, sores, wounds, hair loss, genital ulcers, hemorrhoids, boils, inflammation, and as a laxative. In India, the whole leaves and the fresh gel have been used as a laxative, to improve appetite and digestion, to promote menstrual flow, and to destroy and expel intestinal worms. In the seventh century, aloe gel was used in Asia for inflammatory skin conditions and sinusitis. Aloe latex (taken from the bundle sheath cells inside the leaf) has been used for its laxative effect; however, in 2002, the FDA banned the use of aloe latex in over-the-counter drug products in the United States (Moghaddasi and Kumar, 2011).

With numerous properties, *Aloe* is among the most well-known herbs. This member of *Liliaceae* is similar to cactus in appearance and mostly grows in arid regions of Asia and Africa (Boudreau and Beland, 2006). The middle major parts of *Aloe vera* leaves consist of the gel. Previous studies discovered different properties of *Aloe vera* gel, including wound healing, anti-parasitic, anti-viral, antifungal and anti-bacterial properties (Boudreau and Beland, 2006; Reynolds and Dweck, 1999). An important *Aloe vera* gel complex which has received attention from researchers is the polysaccharide acemannan – a mannose polymer (Reynolds and Dweck, 1999). Studies revealed that properties of *Aloe vera*, including wound healing, immunomodulatory, and antibacterial properties, may stem from acemannan (Mascolo et al., 2004). Studies performed on the effects of *Aloe vera* gel and of polysaccharide contained in *Aloe vera* (acemannan) on the broilers have shown that *Aloe vera* gel can improve the immune response in broilers (Chinnah et al., 1992; Valle-paraso et al., 2005).

Apart from polysaccharides, miscellaneous bioactive constituents have been identified

from the leaves and roots of Aloe plant. These Aloe compounds belong to different classes such as alkaloids, anthraquinones, saccharides, enzymes, amino acids, inorganic mineral, etc. (Franz et al., 2005). In regards to the healing properties, many researches have demonstrated that the mucilaginous polysaccharides contained in the clear pulp of Aloe leaf are the major ingredient responsible for the healing. However, new evidence has shown that emodin, one of the derivatives of anthraquinones produced by superficial pericyclic cells, is also capable of promoting the repair of rats' excisional wounds via stimulating tissue regeneration. This is a supporting evidence to the claim that the healing function of Aloe plant is essentially a result of the synergistic mode of action of many bioactive compounds, rather than one single "magic bullet".

Aloe gel has demonstrated wound healing (Davis, 1989; Heggers, 1993), anti-inflammatory (Vazquez et al., 1996), antiviral (Saoo et al., 1996), spermicidal (Fahim and Wang, 1996), and gastroprotective (Danhof, 1991) properties. It has also shown immune-stimulating (Zhang and Tizard, 1996) and cholesterol-lowering activity. Aloe gel is thought to speed recovery from wounds by enhancing the activity of macrophages and fibroblasts (Heggers, 1993). A recent study showed aloe to be more effective than conventional treatments for burns, frostbite, and intra-arterial damage from intravenous drug abuse, as well as tissue injuries from electrical shocks (Heggers, 1993). Aloe gel also helped speed the rate of wound closure and the resistance of the healed wound to tearing. Its wound-healing activity has been partially attributed to the polysaccharide, mannose-6-phosphate.

It has been suggested that aloe works as an anti-inflammatory when used topically and when taken orally (Davis, 1989). An animal study examined the anti-inflammatory activity of extracts derived from aloe gel. The researchers concluded that the action may be due to inhibition of the arachidonic acid pathway through cyclooxygenase (Vasquez, 1996). Another study inhibited edema in mice by 37 percent and attributed the activity to the sterol compounds in aloe, especially lupeol. Antiviral activity was shown in an in vitro study using a purified extract from the gel of Aloe barbadensis. The main activity against cytomegalovirus was shown 12-36 hours after infection (Saoo, 1996). An in vitro study using zinc acetate and lyophilized Aloe barbadensis (7.5 and 10 percent) demonstrated an antiviral and spermicidal effect. The authors concluded that it may be useful as a contraceptive, especially significant in preventing the transmission of HIV.

Spermicidal activity from Aloe was thought to be due to microelements (boron, barium,

calcium, chromium, copper, iron, potassium, magnesium, manganese, phosphoru, and zinc) that immobilize the tails of sperm without causing vaginal irritation (Fahim and Wang, 1996). Gastroprotective properties were shown in several animal studies. When aloe gel was administered to rats prior to ulcer-inducing stress, the number of ulcers decreased by 80 percent. After developing ulcers, the animals given aloe gel recovered three times faster than the control animals (Danhof, 1991). Another animal study demonstrated similar results. The triterpenoids contained in aloe were found to protect against the formation of gastric ulcers. Research on immune stimulation in mice has indicated that acemannan, a polysaccharide within aloe, has demonstrated dose-dependent macrophage activation (Zhang and Tizard, 1996). When given orally to animals, mannans have also been shown to inhibit cholesterol absorption and lower cholesterol. The pharmacological difference between aloe gel and aloe latex is that the gel does not contain any anthraquinone compounds and does not, therefore, exert any laxative action (Newall et al., 1996). Anthraquinones induce secretion of water and electrolytes into the lumen of the gut and inhibit the absorption of electrolytes and water by the colon. This action in turn activates peristalsis (Blumenthal et al., 1998; Bradley, 1992). Aloe-emodin, a degradation product of Bglycosides in the colon, is the laxative metabolite (Blumenthal et al., 1998). Animal studies have found that aloe-emodin and an alcoholic extract of aloe possess antitumor and anticancer activity. In vitro research has reported that aloctin A could induce cells cytotoxic to syngenic and allogenic tumor cells (Newall et al., 1996; Wren, 1988). However, the National Cancer Institute is no longer studying the latex as an anticancer treatment.

The present study aimed to evaluate the influence of supplying the diet of pregnant buffalo cows with *Aloe arborescens* on the colostrum immunoglobulin content and on the *in vivo* performance of the calves born from these animals.

2.2.2. *Material and methods*

Animals and dietary treatments

The trial was carried out in 2010 on agro-zootechnical farm situated at 47 m a.s.l. in Southern Italy (Sant'Angelo in Theodice, Cassino, FR), where about 200 Italian Mediterranean buffaloes are bred.

Sixteen Italian Mediterranean pluriparae buffalo cows were divided into two groups (homogeneous for parity, body condition score and milk yield in the previous lactations) during the last two months of pregnancy. Both groups were fed a diet (CP: 12 % DM; 0.75 MUF/kg DM) constituted by oat straw, corn silage and concentrate. Group "Aloe" received a supplementation of 50 g/day/head of a commercial product (Vigoorsan® NUTRIZOO s.a.s., Italy) containing *Aloe arborescence*, while group "Control" was the control.

Colostrum analysis

Within one hour from calving, samples of colostrum were collected from each subject and divided into two aliquots. The first was analyzed for fat, protein and lactose (Milko Scan 133B, Foss Matic, Hillerod, Denmark, calibrated with an appropriate standard for buffalo milk). The second aliquot of colostrum was centrifuged at 4000 rpm to remove fat and, then, ultracentrifuged at 30000 rpm, the intermediate layer was used from IgG assay. Serum IgG concentration was determined by use of a commercially available radial immunodiffusion according to the manufacturer's specifications (Bethyl Laboratories, Montgomery, USA). Briefly, 5 μL of serum was added to 1 well of a 48-well plate containing anti-bovine IgG antiserum dissolved in 1.5% agarose in tris-buffered saline solution and 0.1% sodium azide. Three reference standards (1.25, 5, and 10 mg/mL) included in the kit were tested concurrently with each sample, therefore, all samples were diluted 1:10. The plate was incubated at room temperature (23°C) for 24 hours, and the precipiting ring diameter was measured. The IgG concentration of test samples was determined by comparing precipiting ring diameter for test samples to a semilog plot generated from results for the reference standards.

Calves in vivo performances

After 6 days of colostrum administration, the 16 calves, born form the buffalo cows of both groups, received 6 l/head/d of acidified milk replacer (in the ratio of 180 g/l of water) until 56 d. Subsequently, the replacer amount was gradually decreased, whilst administering the same volume. Roughly chopped alfalfa hay and weaning concentrate were available from the fifth week; corn silage was administered starting from 70 d.

All the animals were weighed at the birth and thereafter every week until weaning

(about 90 days). The parameters referred to living animals [body weight, daily weight gains, biological efficiency of growth (BEG = $DWG/BW^{0.75}$] were calculated from the data of the individual curves of growth (Pilla et al., 1987; Pilla, 1991) using the quadratic ($y = = a+bx+cx^2$) equation.

Statistical analysis

Concerning colostrums IgG content, one-way ANOVA (SAS, 2000) was used to detect statistical differences between groups (Control vs. Aloe).

In vivo performance data were statistically processed to determine the effect of Aloe addition (Aloe vs. Control) using the SAS (2000) GLM procedure, according to the following model:

$$y_{ij} = \mu + Treat_i + \varepsilon_{ij}$$

where: y = dependent variable, μ =mean, PS= treatment (i = Aloe, Control) and ε = error.

2.2.3. *Results and discussion*

Colostrum analysis

Chemical composition and immunoglobulin concentration of colostrum collected from groups Aloe and Control is reported in table 27. Buffalo colostrum was rich in fat and proteins (especially IgG) in agreement with previous results (Lombardi et al., 1996), however, no significant differences were registered between groups, even if proteins were higher for group fed diet supplemented with *Aloe arborescens.*

Concerning immunoglobulin concentration, animals of both groups included in the trial produced a good quality colostrums; indeed, value of 60 mg/ml registered within one hour from calving is considered a threshold for discriminate scarce or sufficient concentration (Lombardi et al., 2001). Despite that, colostrum of group Aloe showed a significant ($P<0.05$) IgG increase (78.54 ± 8.3 vs. 71.28 ± 9.0 mg/ml); such results suggest that *Aloe arborescens* supplementation may increase the immunological properties of colostrum thus resulting in improving passive transfer in newborn calves. If true, it is supposable that, in case of dams producing a medium or low quality colostrum, the Aloe supplementation may increase its immunological properties thus

reaching a quality still acceptable to ensure passive transfer in calves. As a whole, present results were in agreement with those of our previous study (Lombardi et al., 2001). Dang et al. (2009) reported lower IgG concentration (54.0 mg/ml) in buffalo colostrum; however, the study was carried out on a different buffalo breed (Murrah). The mechanism by which Aloe may lead to such a result needs further studies, however, it has to be underlined that cell surface polysaccharides (Sironi et al., 1990) when recognized by pattern recognition receptors (PRRs) are effective stimuli for the activation of quiescence macrophages and other immune cells (Gordon, 2002). These immunostimulatory substances are generally named polysaccharide biological response modifiers (BRMs). Polysaccharide BRMs are not limited to microbial origin but also to botanical origin. According to the sugar compositions, there are three major groups of polysaccharide BRMs, which are β-1,3-Dglucans, α/β-1,4-mannan and highly branched polysaccharide of very heterogenous monosaccharide compositions. β-1,3-D-Glucans are mainly derived from cell wall or cytoplasmic reserve of fungus (Garner and Hudson, 1996; Ni et al., 2004). α/β-1,4-mannan is mainly derived from yeast cell wall and freshly layer of Aloe leaves (Nose et al., 1998). In particular, Leung et al. (2004) described the isolation and characterization of the polysaccharide BRM, PAC-I, which was purified from *Aloe vera* L. var. *chinensis* (Haw.) Berg., which is a variant of *Aloe vera barbadensis* Miller and has wide occurrence in China. PAC-I was determined to have mannose as the major monomeric unit. β-1,4-D-Linked mannose contributes to the polysaccharide main skeleton. The molecular weight of PAC-I was 10,000 kDa. PAC-I was demonstrated to exhibit potent stimulatory effects on B and T lymphocytes.

Successively, the same authors (Liu et al. (2006) reported that the administration of PAC-I into allogeneic mice stimulated systemic TNF-α production in a dose-dependent manner and prolonged the survival of tumor-bearing mice. PAC-I is thus a potent stimulator of murine macrophage.

In vivo animal performance

As showed in table 28 the buffalo young calves born by mother of Aloe group showed better *in vivo* performance; in particular, the DWG for all the period 7-90 days was significantly ($P<0.01$) higher in Aloe group compared to the control (0.731 vs. 0.573). The body weight improved in Aloe group after the first month of life of the calves (figure 14) while the BEG was always better.

The different growth performances between groups cannot be explained either by chemical composition of colostrum, which was similar notwithstanding the slightly higher protein found for group fed diet supplemented with *Aloe arborescens*, or by feeding of calves until 90 days, which was the same for all the animals. In addition, no clinical signs was registered in calves of Control group.

Thus, we can formulate the hypothesis that a colostrums richer in IgG is better from the nutritional point of view. Indeed, immunoglobulins in colostrum is highly linked to a number of growth and maturation factors such as somatomedins (IGF-1, IGF-2), somatotrophin, fibroblast growth factor, transforming growth factor (TGF), insulin, platelet derived growth factor (PDGF) and epidermal growth factor (EGF) which form a powerful combination (Oda et al., 1989; Ginjala and Pakkanen, 1998). These naturally occurring substances have been shown to enhance the synthesis of DNA, RNA and protein, while at the same time inhibit breakdown of protein (Ballard et al., 1982; Ginjala and Pakkanen, 1998).

There are receptors for these compounds throughout the intestinal tract and they are postulated to be mediators of intestinal growth and development (Montaner et al., 1999). They are known to increase cell mass of intestine, influence the composition of absorptive surfaces and may be involved in stimulating wound healing (Hardin et al., 1993). Maturation and proliferation of intestinal cells result in increased absorption of electrolytes and nutrients from intestine (Opleta-Madsen et al., 1991; Alexander and Carey, 1999).

2.2.4. *Conclusions*

Our results suggest that *Aloe arborescence* supplementation may improve buffalo colostrum immunological properties thus leading to a higher passive immunization of calves.

3. GENERAL DISCUSSION

The market interest in buffalo meat has progressively increased also in the developed countries due to the negative trend towards bovine meat consumption, which has recently worsened due to the BSE scare.

In Italy the issue of a Code for buffalo meat production is in progress. A very important article will concern the commercialization only of subjects whose weight gain result in the physiologic range, the latter based on the data obtained from the trial reported in the first part of this thesis. In fact, it is thought that daily weight gains largely above or below that previously indicated, could be due to the use of outlaw growth promoters and to animal sufferance, respectively. This to guarantee high quality of meat that is the only way to develop the market, due to the not very favourable growth and mainly dressing percentage of buffalo.

In fact, it needs to underline the nutritive characteristics of buffalo meat. In this field particularly interesting are the results on the fatty acids profile and cholesterol content of buffalo meat. The data allow the following considerations:

- the cholesterol content results lower than those found for the Italian bovine bred specialised for meat production. Other authors that investigated on the cholesterol content of buffalo meat got values superimposable (46 mg/100 g, Sinclair et al., 1982) or even lower than ours (32 mg/100s g, Yadava and Singh, 1974). Finally, Cutrignelli et al. (1996), found a not uniform distribution of the cholesterol content. These authors, in fact, reported very different values in groups of buffalo young bulls bred in the same manner: 32.2 ±s 2.5 and 50.6 ±s 2.8 mg/100 g;
- the contents of myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids result significantly lower than those recorded by Poli et al. (1996) for young bulls of Chianina bred. To the light of the both high atherogenic and thrombogenic activity of the first two fatty acids and of that only thrombogenic of the stearic acid, we can attribute a favourable judgment to buffalo meat from the dietetic-nutritional point of view. In fact, although the contents of oleic acid and of PUFA of the ω-6 and ω-3 series are not particularly high, both the atherogenicity (AI) and the thrombogenicity

(TI) index were very low.

In the first trial of the present PhD thesis it has been demonstrated that feeding growing buffalo with diet without soybean does not affect either *in vivo* performances (body weight, daily weight gain and biological efficiency of growth, feed conversion index) or nutritional characteristics of meat. This is very interesting, due to the public concerns about genetically modified feed, as large part of soybean nowadays is.

The second trial confirmed the influence of good quality colostrums on the calves growth. Indeed, the addition of *Aloe arborescens* to the mother's diet during the last period of pregnancy, significantly increased the immunoglobulin content of colostrums. This result, in addition to determine and adequate passive transfer of immunity, very probably also determined the higher growing performances of calves due to the link of IgG to a number of growth and maturation factors able to increase absorption of nutrients from intestine.

4. TABLES AND FIGURES

Table 1.

Total Buffalo population in the world in 2008

Country	Head	Country	Head
Afghanistan	-	Malaysia	131,000
Albania	120	Mauritius	25
Argentina	-	Mexico	-
Islamic Republic of Iran	630,000	Federated States of Micronesia	140
Australia	-	Myanmar	2,923,568
Austria	-	Nepal	4,496,507
Azerbaijan	298,975	New Zealand	-
Bangladesh	1,260,000	Nigeria	-
Bhutan	2,000	Pakistan	29,000,000
Bosnia and Herzegovina	13,000	Paraguay	-
Brazil	1,146,798	Philippines	3,338,570
Brunei Darussalam	4,580	Portugal	-
Bulgaria	8,968	Republic of Korea	-
Cambodia	746,207	Romania	-
Canada	-	Russian Federation	14,363
China	23,271,909	Saudi Arabia	-
Colombia	-	South Africa	-
Democratic People's Rep. Korea	-	Yugoslav Rep. Macedonia	640
Egypt	4,052,649	Suriname	1,000
France	-	Syrian Arab Republic	6,000
Georgia	17,200	Tajikistan	15,000
Germany	-	Thailand	1,699,469
Greece	1,643	Sri Lanka	318,530
Guam	70	Timor-Leste	102,216
Hungary	-	Trinidad and Tobago	5,700
India	105,127,000	Tunisia	-
Indonesia	1,930,716	Turkey	84,705
Armenia	497	Ucraine	-
Iraq	285,537	United Arab Emirates	-
Israel	-	United Kingdom	-
Italy	294,000	United States of America	-
Japan	-	Uruguay	-
Lao People's Democratic Rep.	1,155,000	Venezuela (Bolivarian Rep. of)	-
Kazakhstan	10,000	Viet Nam	2,897,700
Jordan	100	TOTAL	185,292,102

Source: FAOSTAT, FAO Statistics division (2010).

Table 2.

Buffalo's number (N) slaughtered and annual percentage increase (PI) by continent (thousand)

		1961/1970	1971/1980	1981/1990	1991/2000	2001/2009
Asia	N	7,167/ 8,885	9,140/ 11,007	11,338/ 15,040	15,506/ 18,897	19,421/ 22,414
	PI, %	23.97	20.42	32.65	21.86%	15.41
AFRICA (only Egypt)	N	496/ 695	731/ 934	956/ 1,215	1,250/ 1,640	1,073/ 1,550
	PI, %	40.12	27.77	27.09	31.2	44.45
Europe	N	80/ 45	0.041/ 29	25/ 10	11/ 9	6/ 11
	PI, %	-43.75	-29.26	-60	-18.18	83.33
World	N	7,744/ 9,626	9,912/ 11,971	12,32/ 16,265	16,767/ 20,547	20,501/ 23,976
	PI, %	24.30	20.77	32.02	22.54	16.95
Meat	tons	1,071/ 1,313	1,341/ 1,605	1,680/ 2,266	2,336/ 2,834	2,792/ 3,307
	PI, %	22.59	19.68	34.88	21.31	18.44

Source: FAOSTAT, FAO Statistics division (2010).

Table 3.

Number of buffalo head buffalo farms in Campania region

Number of males	18,159
- age 0 - 7 months	3,247
- age 7 - 10 months (“asseccaticci”)	1,803
- age 10 - 12 months (“annutoli”)	781
- age 12 - 24 months (“annutoli”)	4,820
- age higher than 24 months (Bulls)	7,508
Number of females	253,012
- age 0 - 3 months (Calves)	5,980
- age 3 - 6 months (Weaned calves)	7,867
- age 6 - 12 months (Heifer “manzette”)	16,438
- age 12 - 18 months (Heifer “manze”)	17,413
- age 18 - 36 months (Heifer “giovenche”)	45,730
- age 36 - 51 months (Primiparous)	36,698
- age 51 - 81 months (Pluriparous until 3rd delivery)	55,165
- age more than 81 months (Pluriparous from 3rd delivery)	67,721
Total	271,171

Source: www.statistiche.izs.it

Table 4.

Mean meat intake (g/day) by recall day, age, body mass index (BMI), smoking, educational level and sports activity, adjusted with and without inclusion of total energy intake, in women and men participating in the European Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

			Total meat intake (g day^{-1})							
			Without energy adjustment				With energy adjustment			
			Women		Men		Women		Men	
Factor	Women (*n*)	Men (*n*)	Mean†	SEM	Mean†	SEM	Mean†	SEM	Mean†	SEM
Recall day										
Monday	3960	2141	86.2^{a}	2.4	137.5^{a}	4.9	89.7^{a}	2.3	137.8^{a}	4.5
Tuesday	4007	2212	85.3^{a}	2.4	128.5^{b}	4.8	88.5^{a,c}	2.3	127.8^{b}	4.5
Wednesday	3724	2064	81.4^{b,c}	2.4	132.2^{b}	4.9	83.6^{b}	2.3	131.1^{b}	4.6
Thursday	3205	1937	82.1^{a,b}	2.5	126.9^{b}	5.0	84.7^{b,c}	2.4	125.8^{b}	4.6
Friday	2178	1456	73.9^{c}	2.7	130.7^{a,b}	5.4	74.4^{c}	2.6	122.7^{b}	4.9
Saturday	2756	1525	93.0^{d}	2.6	158.1^{c}	5.3	88.0^{a}	2.5	138.5^{a}	4.9
Sunday	3094	1696	104.4^{e}	2.5	157.3^{c}	5.1	100.6^{e}	2.4	148.5^{c}	4.7
Age (years)										
35– < 45	2231	1106	97.1^{a}	2.8	154.0^{a}	5.6	94.8^{a}	2.7	139.0^{a}	5.2
45– < 55	8597	3953	88.3^{b}	2.1	145.7^{b}	4.5	88.2^{b}	2.0	137.7^{a}	4.1
55– < 65	9003	5910	83.6^{c}	2.1	133.6^{c}	4.4	84.8^{c}	2.0	130.6^{b}	4.0
65–74	3092	2062	77.5^{d}	2.5	121.7^{d}	5.4	80.5^{d}	2.4	125.3^{b}	4.9
BMI (kg m^{-2})										
< 20	1385	139	75.9^{a}	3.0	126.7^{a,b}	11.0	72.6^{a}	2.9	117.1^{a}	10.1
20–25	10879	3766	82.8^{b}	2.0	132.5^{b}	3.8	82.2^{b}	1.9	125.4^{a}	3.5
25–30	7363	6882	91.3^{c}	2.1	141.5^{a}	3.5	93.3^{c}	2.0	137.7^{b}	3.2
> 30	3296	2244	96.5^{d}	2.5	154.3^{c}	4.3	100.2^{d}	2.4	152.4^{c}	3.9
Smoking										
Never	13136	4196	84.2^{a}	1.9	135.9^{a}	4.6	83.4^{a}	1.8	130.0^{a}	4.2
In the past	5189	5061	83.8^{a}	2.1	135.2^{a}	4.5	84.2^{a}	2.0	130.6^{a}	4.1
Currently	4135	3558	96.2^{b}	2.2	145.2^{b}	4.6	96.7^{b}	2.1	138.9^{b}	4.2
Education										
None	964	749	80.6^{a}	3.6	148.0^{a}	6.1	82.6^{a}	3.4	142.7^{a}	5.6
Primary school	6221	4344	92.6^{b}	1.5	146.4^{a}	3.7	93.8^{b}	1.5	141.4^{a}	3.4
Technical school	4276	2913	92.2^{b,c}	1.8	139.7^{b}	3.8	92.8^{a,b}	1.8	133.7^{b}	3.5
Secondary school	6297	2092	88.1^{c}	1.5	133.8^{b}	4.1	87.1^{a,c}	1.5	131.9^{b}	3.7
University degree	4782	2804	82.7^{a}	1.7	121.1^{c}	3.8	79.9^{c}	1.6	120.6^{c}	3.5
Sports activity (h week^{-1})‡										
None	4053	2674	88.9^{a,c}	2.2	143.8^{a}	4.6	91.3^{a}	2.1	139.0^{a}	4.2
> 0–2	3827	2255	90.2^{a,c}	2.3	140.8^{a}	4.8	90.7^{a}	2.2	135.3^{a,c}	4.4
> 2–4	4557	1742	86.3^{b,c}	2.3	138.6^{a,b}	5.0	86.3^{b}	2.2	134.1^{a,c}	4.6
> 4–8	3189	1873	84.8^{b}	2.4	138.2^{a,b}	5.0	83.8^{b}	2.3	131.4^{b,c}	4.6
> 8	2969	2188	82.8^{b}	2.7	132.3^{b}	5.3	83.3^{b}	2.6	126.0^{c}	4.9

* Adjusted for recall day, centre, age class, BMI class, smoking, education and sports activity, with or without adjustment for total energy intake (continuous).
† Different superscripts indicate significantly different means within gender, factor and model. Vice versa, means with identical superscripts are not significantly different from each other within gender, factor and model; LSD-test, $P < 0.05$
‡ Without data from Norway.

Source: Linseisen et al. (2002).

Table 5.

World milk production (excluding butter) in 2007

Country	*Tonnes*
India	80,040,899.49
United States of America	78.342.858.40
China	38,354,332.89
Pakistan	27,542,690.63
Russian Federation	24.479.719.29
Brazil	23,691,081.59
Germany	20,358,630.73
France	16,075,183.98
Italy	15,187,073.09
United Kingdom	14,760,971.92
Mexico	12,380,652.02
Turkey	10,126,540.05
Japan	9,739,105.85
Argentina	8,414,375.67
Ucraine	7,995,878.24
Spain	7,818,422.04
Poland	7,569,396.01
Sudan	7,305,184.86
Canada	6,813,863.43
Romania	5,709,858.17
Colombia	5,437,211.09
Netherlands	5,269,734.61
Egypt	4,948,580.09
Australia	4,815,573.98
Iran (Islamic Republic of)	4,789,747.49
Kazakhstan	4,046,276.41
Algeria	3,973,423.39
Uzbekistan	3,856,293.67
Kenya	3,724,293.05
Greece	3,496,792.23
Sweden	3,259,323.66
South Africa	2,848,017.00
Indonesia	2,578,025.99
Bangladesh	2,552,386.23
Belgium	2,511,344.45
Venezuela (Bolivarian Republic of)	2,414,156.22
Saudi Arabia	2,399,547.27
Switzerland	2,372,434.99
Portugal	2,372,351.01
Syrian Arab Republic	2,284,692.36
Czech Republic	2,007,088.52
136 Others countries less than 2.000.000 tonnes	64,302,034.59
World milk Production 2007	558,966,046.65

Source: FAOSTAT Statistics division (2011)

Table 6.

Chemical composition of bovine and buffalo milk as fed and on DM; nutrient intake as a function of intake capacity

	Milk, *g/kg*		Milk, *g/kg DM*		Intake/*100 kg LW*		
	bovine	buffalo	bovine	buffalo	bovine	buffalo	Δ %
DM	122	188	1000	1000	2400	2000	
Protein	31	46	254	245	610	489	+ 24.74
Fat	34	83	279	441	669	883	- 24.24
Lactose	49	49	402	261	964	521	+ 85.03
Ash	8	10	65.6	53.2	157	106	+ 48.11
Ca	1.2	1.9	9.84	10.11	23.61	20.21	+ 16.82
Colloidal Ca	0.8	1.5	6.9	8.09	15.82	16.17	- 2.15
P	0.9	1.1	7.38	5.85	17.70	11.70	+ 51.28
Kcal	673	1238	5515	6580	13239	13160	+ 6.00

Table 7.

Chemical composition (%) of an acidified milk replacer for buffalo calves

Water	Crude protein	Fat	Crude fibre	Ash	N-free extracts
4	24.2	21	0.2	8	42.6

Premix for kg: lisin 2%; methionin 0.6%; cistin 0.3%; vit. A 62500 IU; vit. D3 7500 IU; vit. E 80 mg; vit. C 120 mg; vit. B1 6 mg; vit. B2 10 mg; vit B6 4 mg; vit B12 50 µg; vit K3 4 mg; niacin 40 mg; D-pantotenic acid 30 mg; colin 600 mg; Fe 80 mg; Mn 70 mg; Zn 50 mg; Co 1 mg; Se 0.15 mg; I 1 mg.

Table 8.

Weights, weight gains, feed conversion index and slaughter yield of buffalo calves at 27 weeks of age, fed with milk replacer (crude proteins: 20.89% and fat: 25.16% on DM basis)

	Male	Female
Initial weight, *kg*	47.89	44.88
Final weight, *kg*	221.56	221.13
Total weight gain, *kg*	173.67	176.25
Daily weight gain kg	0.919	0.933
kg milk/kg growth	1.74	1.71
Slaughter yield %	59.72	58.60
Deposit and kidney fat %	1.45	1.42
Total fat %	11.95	12.56

Source: Romita and Dias da Silva (1975).

Table 9.

Performance of buffalo calves fed milk replacers for bovine or for buffaloes

	IW	WW	WA	TDWG	ADWG	BDWG	CDWG	FCI
	kg	*kg*	*day*	*kg*	*kg*	*kg*	*kg*	DM/DWG
1	49.00^{a}	99.63	94.25^{a}	0.567^{b}	0.659^{b}	0.465^{b}	0.526	2.34^{a}
2	40.75^{ab}	98.63	76.00^{b}	0.831^{a}	1.160^{a}	0.877^{a}	0.497	1.67^{b}
3	37.25^{b}	95.75	73.75^{b}	0.891^{a}	1.178^{a}	0.715^{ab}	0.670	1.58^{b}

1: Bovine; 2: Buffaloes 18%; 3: Buffaloes 22%.
IW: initial weight; WW: weaning weight; WA: weaning age; TDWG: total daily weight gain; ADWG: daily weight gain during phase A; BDWG: daily weight gain during phase B; CDWG: daily weight gain during phase C; FCI: feed conversion index.
Along the column: a,b: $P<0.05$.

Table 10.

Milk replacer intake, growth and slaughtering performance, carcass composition

	C Milk *kg*	C DWG *kg*	F Milk *kg*	F DWG *kg*	T DWG	FCI *kg DM/WG*
R	164.27 a	0.917 a	168.34	1.007 a	0.954 a	1.96 b
V	127.96 b	0.641 b	191.18	0.876 b	0.782 b	2.19 a

	Carcass kg kg	Dressing % %	CS	FS
R	112.9 a	52.28 a	6.1 a	5.6 a
V	89.9 b	51.30 b	5.4 b	4.4 b

	Fore quarter *%*	Meat *%*	Subcut. Fat *%*	Interst. fat *%*	Bone *%*
R	48.9 b	60.35	7.05 a	6.45	23.33 b
V	50.2 a	60.14	5.26 b	5.83	25.25 a

V: cattle milk; R: milk replacer.
C Milk: intake during C phase; C DWG: daily weight gain during C phase; F Milk: intake during F phase; F DWG: daily weight gain during F phase; T DWG: total weight gain (kg); FCI: feed conversion index.
CS: conformation score; FS: fat score.
a,b: $P<0.05$
Source: Failla et al. (2001 a,b)

Table 11.

Meat production in calves bred with buffalo milk(B), cattle milk (V), milk replacer (R) and in pre-weaned calves (S)

	Age *d*	DWG *kg*	M/G	UF/G	SW *kg*	CW *kg*	FQ *kg*	D *%*	M/B
B	117	1.103	6.45	-	152.0	90.3	50.2	59.4	4.24
V	133	0.987	10.57	-	147.8	87.3	50.4	59.1	4.23
R	133	0.973	1.59*	-	150.3	89.1	54.3	59.3	3.83
S	180	0.816	-	2.70	169.7	85.9	66.8	50.6	3.84

*: in powder
DWG: daily weight gain; M/G: kg milk/gain of 1 kg LW; UF/G: forage unity/gain of 1 kg LW; SW: slaughtering weight; CW: carcass weight; FQ: fore quarter; D: dressing; M/B: meat/bone ratio.

Table 12.

Costs (€) of production of young buffalo bulls (350 kg LW) weaned with buffalo milk, milk replacer or cow as nurse

	Buffalo milk	Milk replacer	Cow nurse
Milking phase			
€/kg	1,18	1,86	-
Milk kg/kg DWG	5,17	1,875 (DM)	-
Increment (kg)	200	40	200
Costs (€)			
DWG kg	6,15	3,49	-
Feeding	1229,17	139,44	-
Feeding nurse/day	-	-	3,1
Feeding nurse/day/2 calves	-	-	1,55
Feeding nurse/day for 2 calves for 200 days	-	-	309,87
Phase 40-350 kg LW			
Total feeding	1379,23	466,44	459,94
Total feeding/0,7*	1970,33	666,34	657,05
Total + slaughtering costs	2073,62	769,63	760,35
Nurse (net final sale)	-	-	154,94
Nurse (net final sale/bred calf)	-	-	77,47
Feeding + slaughtering + calf bred by nurse	-	-	837,81
meat (kg)	14,87	5,52	6,01
Live weight (kg)	5,92	2,20	2,39
Compensation			
Nurse/2 calves			116,00
Male + slaughtering	237,99	237,99	237,99
Total compensation	237,99	237,99	353,99
Costs			
Total (net of compensation)	1835,63	531,64	483,82
kg of meat	13,16	3,81	3,47
kg of live weight	5,92	1,52	1,38

* considering the incidence of feeding on the management costs as 70%.
Source: Ferrara and Infascelli (1994).

Table 13.

Meat production by buffalo young bulls different for age and weight

		DWG *kg*	UF/kg DWG	Slaughtering weight *kg*	Dressing *%*
6-12 *months*		0.904	4.67	320.7	52.70
4-16 *months*	NC	0.883	5.59	421.3	56.80
	C	0.851	5.67	405.6	57.88
18-30 *months*	NC	0.519	13.00	616.6	55.90
	C	0.519	13.90	611.3	55.30
30-42 *months*	NC	0.201	37.07	683.2	58.90
	C	0.216	34.67	688.0	60.00

NC: not castrated; C: castrated.
DWG: daily weight gain

Table 14.

Chemical characteristics (%) of buffalo meat

Age	DM	Protein	Fat	Ash
6 *months*	23.0	20.4	1.5	1.1
15 *months*	25.8	21.2	3.6	1.0

Table 15.

Fatty acid profile (%) of buffalo meat

Age	6 months	15 months
C 16	17.71	21.6
C 18	14.86	18.4
C 18:1	39.13	43.3
C 18:2	12.01	5.6
Saturated FA	37.48	43.7
Unsaturated FA	61.50	55.3
Saturated/Unsaturated	0.61	0.79

Table 16.

Chemical composition (%) of meat from Marchigiana and buffalo

	Marchigiana	Buffaloes
Moisture	74.3 ± 0.7	75.8 ± 2.6
Protein	22.0 ± 0.6	21.4 ± 2.7
Fat	2.40 ± 0.6	1.36 ± 0.1
Ash	1.36 ± 0.1	1.37 ± 0.1

Table 17.

Cholesterol content (*mg/100 g of muscle*) and fatty acid profile (*mg/100g of muscle; % of total fatty acids*) of *Longissimus dorsi*

	Marchigiana		Buffaloes	
	m ± sd	%	m ± sd	%
Cholesterol	53.7 7.6		48.8 2.9	
C_{14}	15.3 1.0	1.9	9.9 3.3	1.4
C_{16}	189.3 21	23.0	143.9 12.1	20.0
C_{18}	173.70 16	21.1	117.7 5.2	16.4
$C_{18:1}$	253.0 35	30.8	267.3 2.4	37.2
SFA	383.0 39	46.6	275.7 14.5	38.4
MUFA	255.7 29	31.1	267.9 2.7	37.3
PUFA	182.0 22	22.3	175.0 3.26	24.3
n-6	176.7 19	21.5	170.2 2.39	23.7
n-3	5.27 0.5	0.8	4.74 0.36	0.6
AI	0.57 ± 0.06		0.41 ± 0.04	
TI	1.63 ± 0.13		1.16 ± 0.13	

SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids; AI: Atherogenic Index; TI: thrombogenic index.

Table 18.

Nutritional characteristics of the diet ingredients

	Corn silage	Mixed hay	Concentrate
Content in the diet, *%*	30	20	50.0
Crude protein, *% DM*	7.8	8.0	22.2
Energy, *VFU/kg DM*	0.8	0.6	1.10
Concentrate composition, *%*			
	FB diet	SB diet	
Corn	10	18	
Barley	11	20	
Wheat bran	23	35	
Faba bean	56	-	
Soya bean meal	-	27	

FB: faba bean; SB: soya bean.

Table 19.

Metabolic profile (mean ± standard deviation) at the end of the trial in the two groups

	FB group		SB group	
Glucose, *mg/dl*	53.3	± 15.0	56.1	± 10.9
Urea, *mg/dl*	15.6	± 4.10	13.8	± 3.60
Creatinine, *mg/dl*	0.80	± 0.20	0.80	± 0.20
Cholesterol, *mg/dl*	42.6	± 11.4	42.8	± 11.1
Triglycerides, *mg/dl*	11.5	± 2.90	15.6	± 4.70
GOT-Aspartate, *UI/l*	83.9	± 24.5	80.1	± 28.7
GPT-Alanine, *UI/l*	30.9	± 8.60	31.0	± 9.60

FB: faba bean; SB: soya bean.

Table 20.

Parameters (mean ± standard deviation) of individual growth curve of the two groups

	a	b	c	R^2
FB group	33.66 ± 0.34	0.372 ± 0.13	0.0009 ± 0.0005	0.9977
SB group	36.47 ± 8.02	0.370 ± 0.23	0.0010 ± 0.001	0.9962

Table 21.

Infra-vitam performance at ages and periods for the two groups

Age, *d*	BEG, *WG/kg$^{0.75}$ BW*			Period, *d*	FCI DM		
	FB	SB	Prob.		FB	SB	Prob.
90	23.15	22.02	NS	90-120	3.551	3.805	NS
120	21.51	20.92	NS	120-150	4.002	4.086	NS
150	20.19	20.05	NS	150-180	4.459	4.427	NS
180	19.11	19.27	NS	180-210	4.918	4.801	NS
210	18.18	18.56	NS	210-240	5.378	5.196	NS
240	17.38	17.90	NS	240-270	5.838	5.603	NS
270	16.67	17.29	NS	270-300	6.297	6.017	NS
300	16.05	16.73	NS	300-330	6.756	6.436	NS
330	15.49	16.21	NS	330-360	7.213	6.858	NS
360	14.98	15.72	NS	360-390	7.669	7.282	NS
390	14.52	15.28	NS	390-420	8.124	7.707	NS
420	14.10	14.86	NS	420-60	8.577	8.132	NS

FB: faba bean; SB: soya bean
BEG: biological efficiency of growth; WG: weight gain; BW: body weight; FCI: feed conversion index; DM: dry matter.
NS: not significant.

Table 22.

Measurements (mean ± standard deviation) *in vivo* and at slaughter in the two groups

In vivo measurements					
	FB group		SB group		Prob.
Width of pelvis, *cm*	45.25	± 2.36	± 1.50	3.92	NS
Width of chest, *cm*	38.00	± 2.94	± 1.71	5.79	NS
Height at withers, *cm*	118.0	± 3.16	± 5.32	19.13	NS
Height at pelvis, *cm*	120.8	± 2.22	± 2.94	6.79	NS
Round circumference, *cm*	167.8	± 3.50	± 7.14	31.6	NS
Length of rump, *cm*	37.00	± 2.45	± 2.58	6.33	NS
Body length, *cm*	117[A]	± 5.86	± 10.42	78.9	**
Depth of chest, *cm*	60.25	± 1.71	± 6.38	21.8	NS
At slaughter					
Length of leg, *cm*	58.75	± 2.99	± 1.50	5.58	NS
Length of carcass, *cm*	108.8	± 4.03	± 6.24	27.6	NS
Width of leg, *cm*	35.50	± 0.58	± 2.16	2.50	NS
Depth of chest, *cm*	36.50	± 4.43	± 2.63	13.3	NS
Thickness of leg, *cm*	20.75	± 0.50	± 2.16	2.46	NS

FB: faba bean; SB: soya bean.
Along the row: A,B: P<0.01. **: P<0.01; NS: not significant.

Table 23.

Measurements (mean ± standard deviation) at the dissection in the two groups

	FB group		SB group		Prob.
Slaughter weight, *kg*	343	± 26.5	355	±13.8	NS
Net weight, *kg*	322	± 24.9	334	± 12.9	NS
Hot dressing out, *%*	50.7	± 1.14	50.5	± 2.66	NS
Net hot dressing out, *%*	53.9	± 1.21	53.7	± 2.83	NS
Net cold dressing out, *%*	50.3	± 2.24	50.2	± 3.44	NS
Sample cut					
Bone, *%*	24.63	± 12.96	24.38	± 2.52	NS
Fat, *%*	22.90	± 5.45	20.07	± 1.94	NS
Meat, *%*	52.46	± 9.79	55.55	± 3.04	NS
Meat/bone	2.59	± 1.22	2.30	± 0.34	NS

FB: faba bean; SB: soya bean.
NS: not significant.

Table 24.

Chemical composition and cholesterol content in the groups and in the muscles

	Fat	Moisture	Protein	Collagen	Ash	Cholesterol
	%	*%*	*%*	*%*	*%*	*mg/100 g*
FB group	1.82^{b}	75.90	21.04^{b}	1.73^{a}	0.76	32.2^{b}
SB group	1.98^{a}	75.73	21.28^{a}	1.63^{b}	0.57	33.7^{a}
IP	2.17^{A}	76.46^{A}	20.58^{B}	1.77^{a}	0.33^{B}	32.8
ST	1.47^{B}	75.53^{B}	21.56^{Aa}	1.65^{b}	0.94^{A}	33.7
LT	2.07^{A}	75.47^{B}	21.33^{Ab}	1.62^{b}	0.72^{A}	32.4
Diet effect	*	NS	*	*	NS	*
Muscle effect	***	***	***	*	***	NS
Interaction Diet*Muscle	NS	NS	NS	NS	NS	NS

FB: faba bean; SB: soya bean.
IP: *Iliopsoas* plus *Psoas minor*; ST: *Semitendinosus*; LT: *Longissimus thoracis.*
Along the column A,B: P<0.01. a,b: P<0.05.
*, ***, NS: P<0.001, P<0.05, not significant, respectively.

Table 25.

Fatty acid profile (g/100 g) in the groups and in the muscles

	Muscles			Groups		Prob.	
	IP	ST	LT	FB	SB	Group	Muscle
C14	1.42^{A}	0.92^{B}	1.31^{A}	1.18	1.25	NS	***
C15-iso	0.19^{A}	0.12^{B}	0.18^{A}	0.16^{b}	0.17^{a}	NS	***
C15-anteiso	0.27^{Aa}	0.19^{B}	0.22^{ABb}	0.20^{B}	0.25^{A}	***	***
C14:1-cis9	0.32^{B}	0.73^{A}	0.35^{B}	0.47	0.47	NS	***
C15	0.37^{Aa}	0.28^{Bc}	0.32^{ABb}	0.29^{B}	0.36^{A}	***	***
C16	21.6^{A}	20.3^{B}	21.0^{AB}	21.0	20.9	NS	*
C17-iso	0.49^{b}	0.57^{a}	0.48^{A}	0.50	0.53	NS	***
C16:1n-7	1.00^{B}	1.35^{A}	1.10^{B}	1.17	1.13	NS	***
C17	1.26^{A}	0.99^{B}	1.25^{A}	1.10^{B}	1.23^{A}	***	***
C17:1-cis10	0.37^{B}	0.49^{Aa}	0.42^{ABb}	0.40^{b}	0.45^{a}	*	***
C18	27.5^{A}	20.0^{B}	27.2^{A}	24.1	25.0	NS	***
C18:1-trans10	0.53	0.64	0.62	0.55	0.64	NS	NS
C18:1-trans11	1.41	1.24	1.29	1.31	1.32	NS	NS
C18:1n-9	27.8^{B}	33.1^{Aa}	31.2^{Ab}	30.1	30.5	NS	***
C18:1-cis11	1.17^{B}	1.48^{A}	1.16^{B}	1.28	1.26	NS	***
C18:2n-6	8.12^{Ab}	9.12^{Aa}	6.47^{B}	7.99	7.89	NS	***
C20	0.21^{A}	0.17^{B}	0.22^{A}	0.19^{b}	0.21^{a}	*	***
C18:3n-6	0.09^{B}	0.14^{A}	0.09^{B}	0.11	0.10	NS	***
C20:1-cis11	0.08^{B}	0.13^{Aa}	0.10^{ABb}	0.10	0.11	NS	***
C18:3n-3	0.56^{b}	0.66^{a}	0.51^{b}	0.57	0.59	NS	***
cis9-trans11 CLA	0.05^{b}	0.06^{a}	0.04^{b}	0.05	0.05	NS	***
trans10-trans12 CLA	0.15^{b}	0.19^{a}	0.17^{ab}	0.17	0.18	NS	NS
C20:2-cis11,14	0.18^{B}	0.25^{A}	0.18^{B}	0.21	0.20	NS	***
C22	0.17^{b}	0.22^{a}	0.18^{b}	0.17^{b}	0.21^{a}	*	*
C20:3n-6	0.55^{B}	0.78^{A}	0.41^{C}	0.63^{A}	0.53^{B}	**	***
C20:3n-3	0.27^{b}	0.33^{Aa}	0.24^{Bb}	0.28	0.28	NS	**
C20:4n-6	2.03^{Ba}	3.10^{Ab}	1.54^{B}	2.41^{a}	2.04^{b}	*	***
C23	0.18^{B}	0.22^{A}	0.16^{B}	0.17	0.21	***	***
C20:5n-3	0.15^{B}	0.28^{A}	0.17^{B}	0.39^{B}	0.49^{A}	NS	***
C24	0.15^{B}	0.21^{A}	0.17^{B}	0.16^{b}	0.19^{a}	*	**
C24:1-cis15	0.45	0.42	0.44	0.39^{b}	0.49^{a}	*	NS
C22:4n-6	0.32^{Ba}	0.45^{A}	0.25^{Ba}	0.36	0.32	NS	***
C22:5n-3	0.44^{B}	0.67^{A}	0.37^{B}	0.54^{a}	0.45^{b}	*	***
C22:6n-3	0.21	0.23	0.22	0.20^{b}	0.24^{a}	*	NS

FB: faba bean; SB: soya bean. IP: *Iliopsoas* plus *Psoas minor*; ST: *Semitendinosus*; LT: *Longissimus thoracis*.

Along the row: A,B,C: P<0.01 and a,b,c: P<0.05. *, ***, NS: P<0.001, P<0.05, not significant, respectively.

Table 26.

Some fatty acids (*g/100 g*) considered of particular nutritional significance in the muscles and in the groups

	Muscles			Group		Prob.	
	IP	ST	LT	FB	SB	Group	Muscle
SFA	53.5^{A}	44.2^{B}	52.5^{A}	49.9^{b}	50.3^{a}	*	***
MUFA	32.8^{C}	39.9^{A}	36.7^{B}	36.3	36.6	NS	***
PUFA	12.91^{b}	16.1^{a}	10.5^{C}	13.4	12.8	NS	***
ω-3	1.61^{B}	2.17^{A}	1.52^{A}	1.79	1.74	NS	***
ω-6	11.1^{B}	13.7^{A}	8.76^{C}	11.4	10.9	NS	***
CLAs	0.20^{Bb}	0.25^{Aa}	0.21^{b}	0.21	0.23	NS	***
						NS	*
PUFA/SFA	0.24^{a}	0.36^{A}	0.20^{Bb}	0.26	0.28	NS	***
ω-6/ω-3	6.95^{a}	6.26^{a}	5.90^{b}	6.44	6.24	NS	*
AI	0.60^{a}	0.42^{b}	0.57^{a}	0.53	0.53	NS	***
TI	1.50^{a}	1.02^{b}	1.51^{a}	1.34	1.35	NS	***

FB: faba bean; SB: soya bean. IP: *Iliopsoas* plus *Psoas minor*; ST: *Semitendinosus*; LT: *Longissimus thoracis*.

SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids; CLA: conjugated linoleic acids;AI: Atherogenic Index; TI: thrombogenic index.

Along the row: A,B,C: P<0.01 and a,b,c: P<0.05. *, ***, NS: P<0.001, P<0.05, not significant, respectively.

Table 27.

Chemical composition and immunoglobulin concentration of colostrum

Groups	Fat *%*	Proteins *%*	Lactose *%*	IgG *mg/ml*
Aloe	15.4 ± 0.7	14.2 ± 0.9	3.1 ± 0.2	78.54^{a} ± 8.3
Control	15.4 ± 0.5	13.7 ± 0.8	3.0 ± 0.8	71.28^{b} ± 9.0

a,b: P<0.05

Table 28.

In vivo parameters in the two groups

	Aloe	Control
Period, *d*	DWG (*kg/d*)	
7-90	0.731^{a} ± 0.128	0.573^{b} ± 0.09
Age, *d*	BEG (*WG/kg0.75 BW*)	
7	82.0^{A} ± 31.5	38.52^{B} ± 9.9
30	51.7^{A} ± 8.8	30.58^{B} ± 6.1
60	36.3^{A} ± 3.5	24.48^{B} ± 3.9
90	28.6^{a} ± 1.9	20.62^{b} ± 2.8

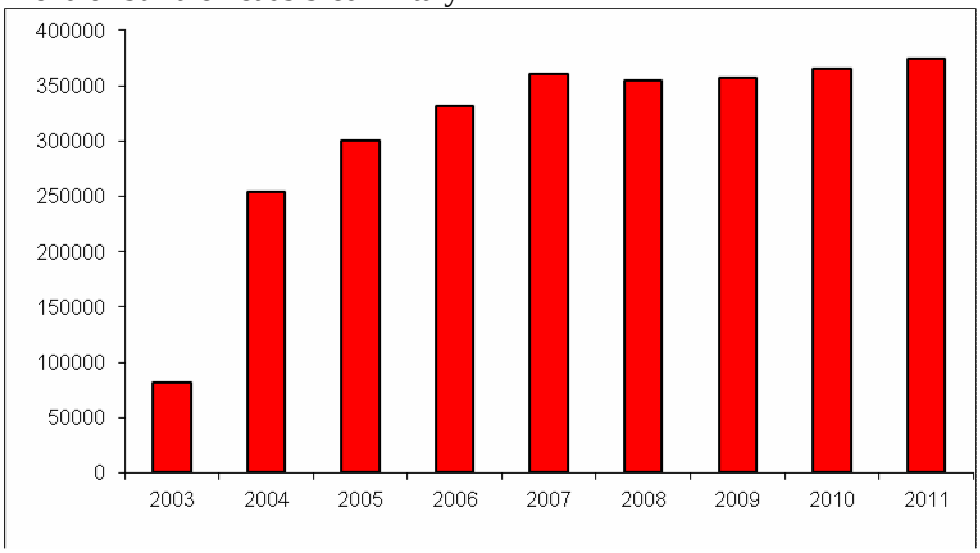
BEG: biological efficiency of growth; BW: body weight; DM: dry matter; WG: weight gain.
A,B: P<0.01; a,b: P<0.05

Figure 1.
River and swamp buffalo

Figure 2.
Italian Mediterranean Buffalo

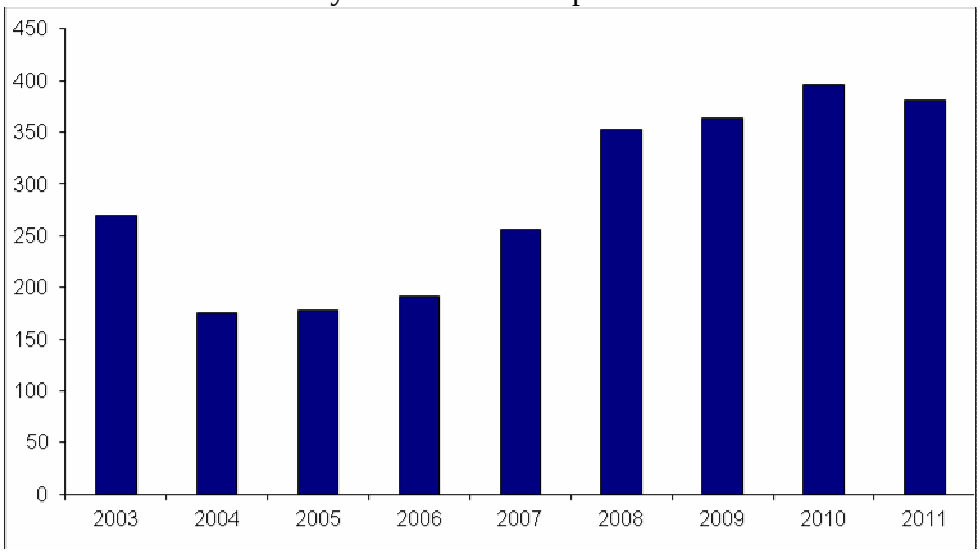
Source: Associazione Nazionale Allevatori Specie Bufalina

Figure 3.
Trend of buffalo heads bred in Italy



Source: www.statistiche.izs.it

Figure 4.
Buffalo heads bred in Italy utilised for meat production



Source: www.statistiche.izs.it

Figure 5.
Mozzarella cheese production

Figure 6.
Coronary Artery Disease

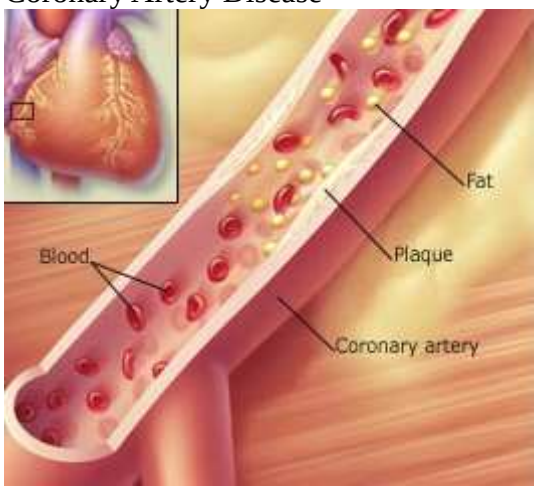
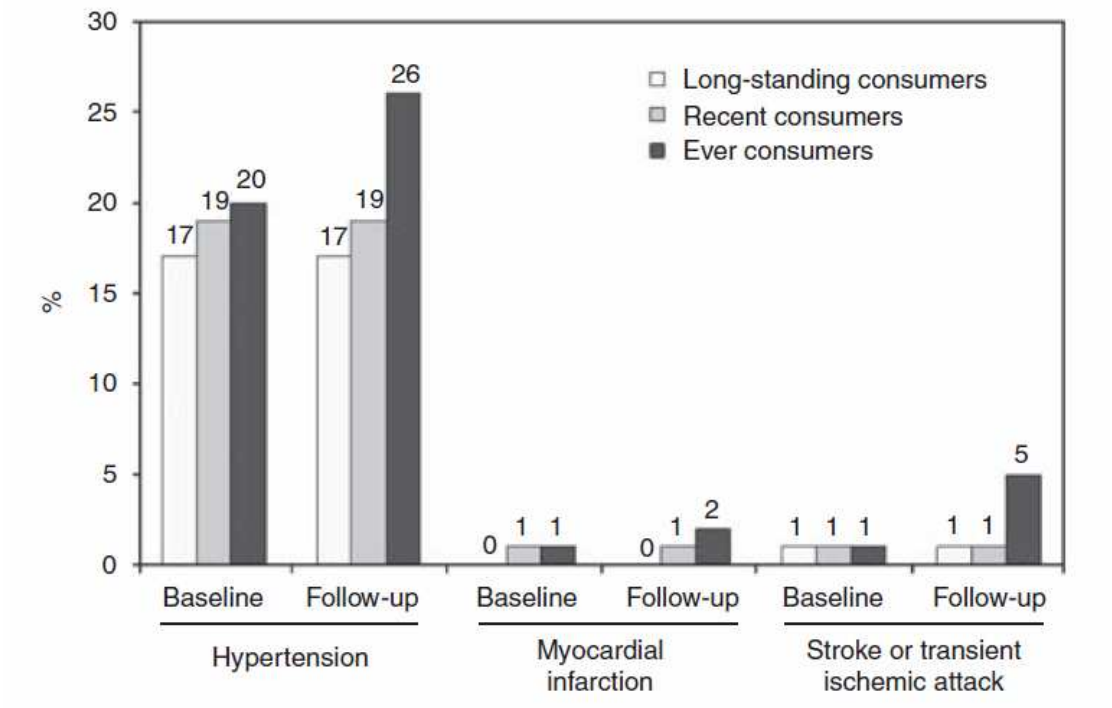


Figure 7.
Relevant of cardiovascular disease to water buffalo meat consumption (P>0.05)



Source: Giordano et al. (2010)

Figure 8.
Scheme of the *in vivo* measurements

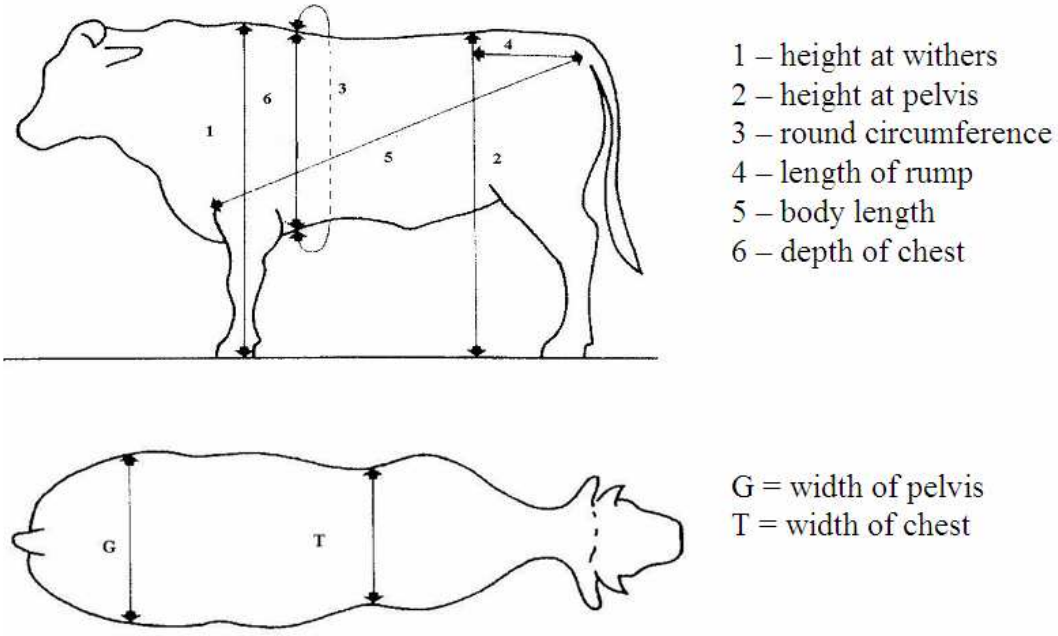


Figure 9.
Scheme of carcass measurements

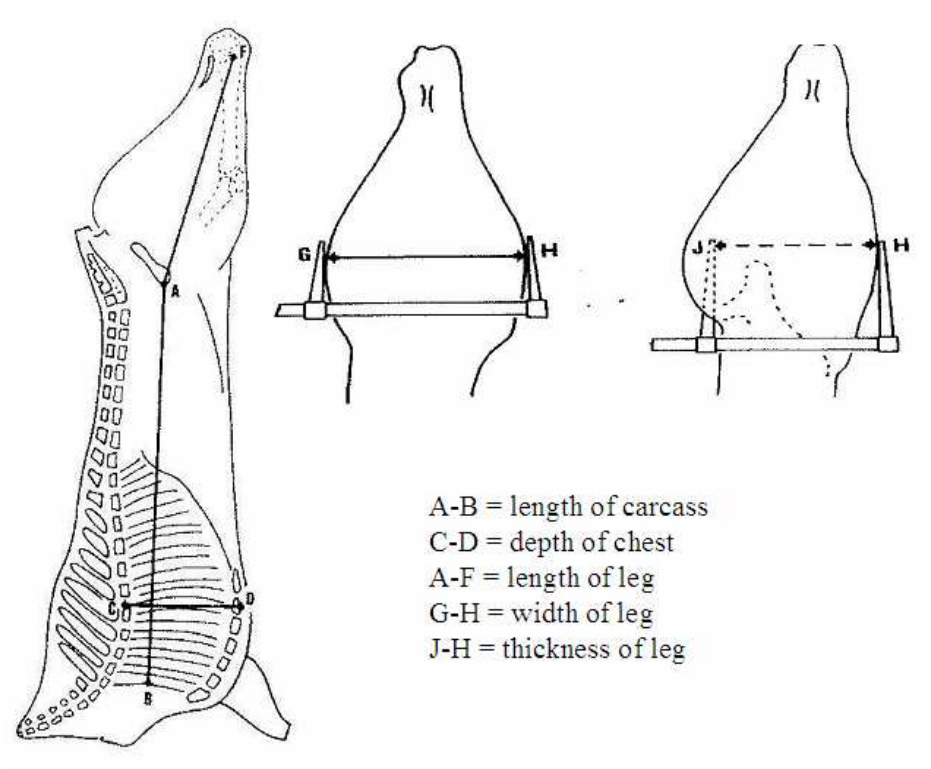


Figure 10.
Infra-vitam performance at ages and periods for the two groups

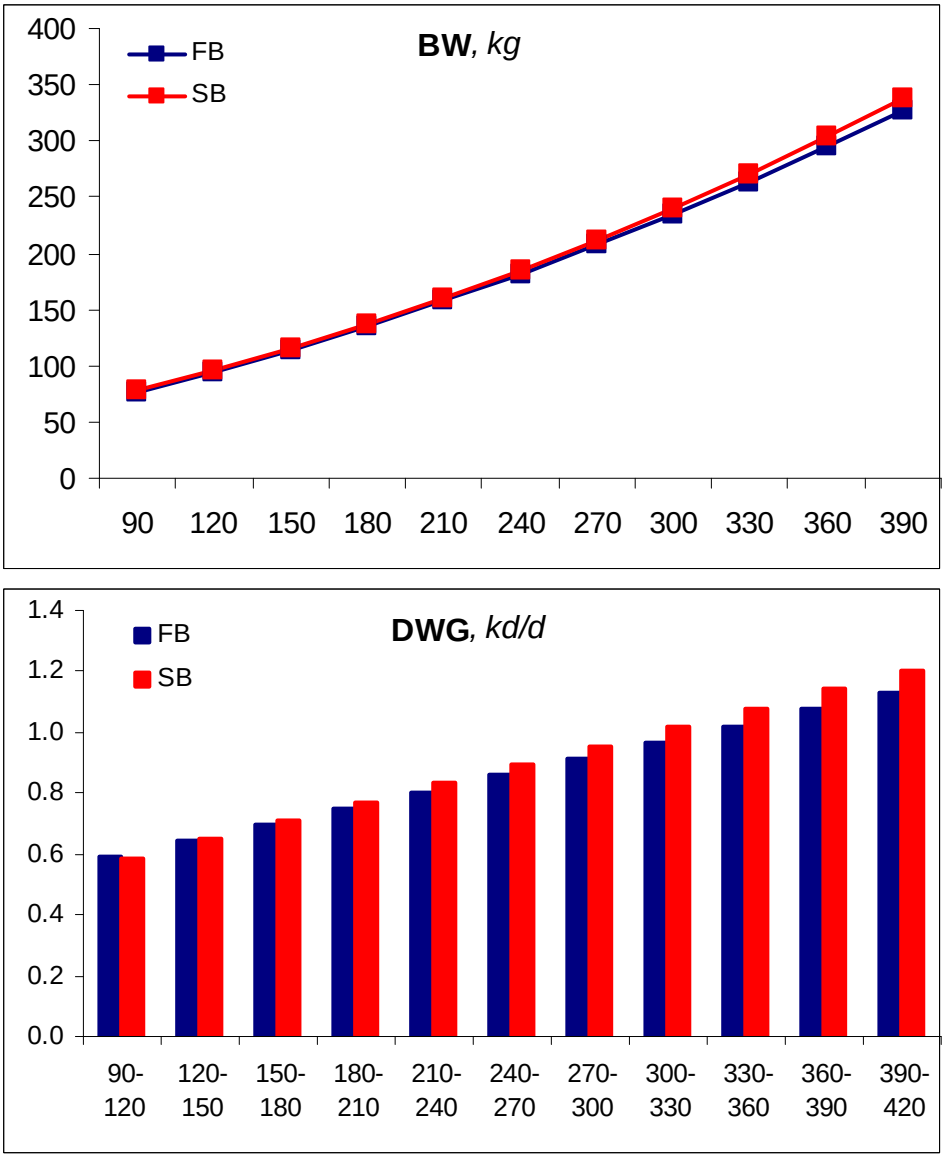
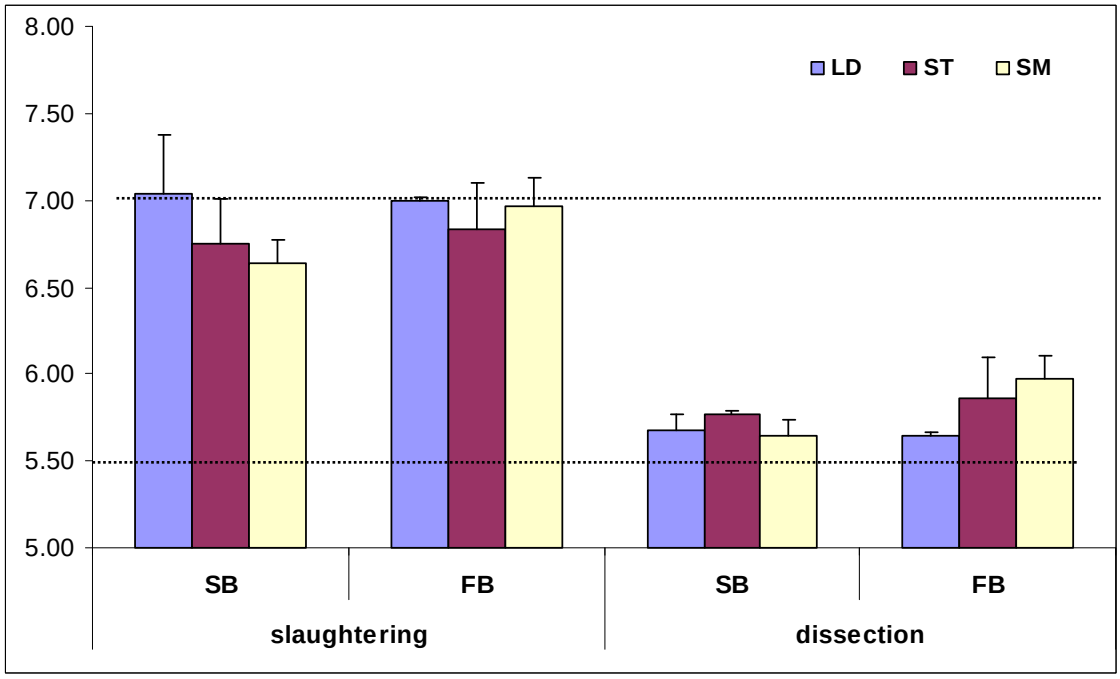
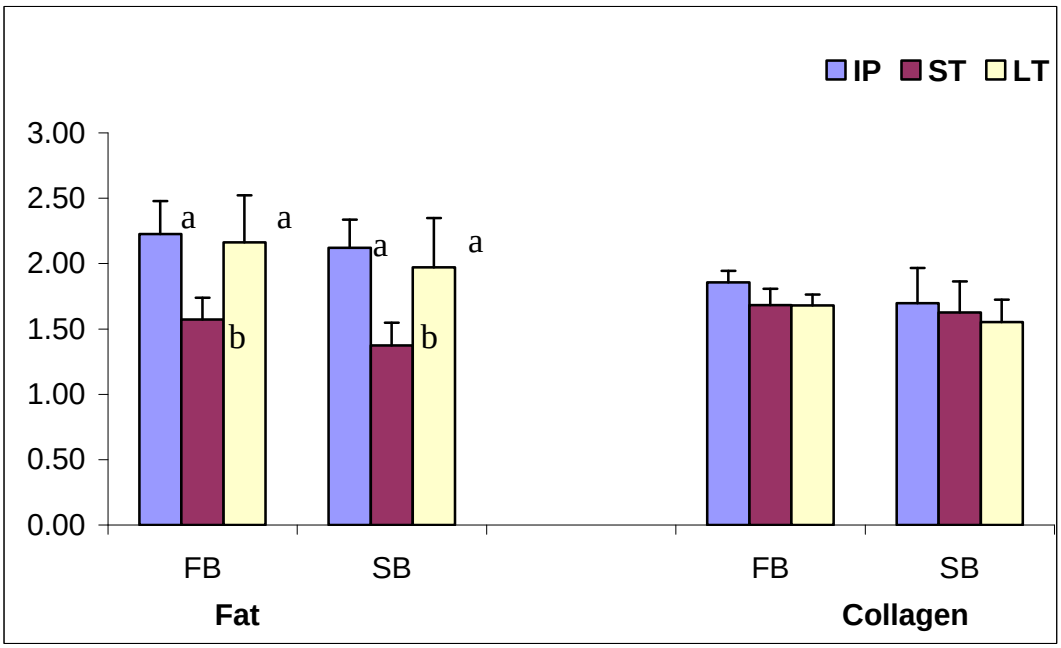


Figure 11.
Means and standard deviation of pH values at slaughtering and dissection in the two groups



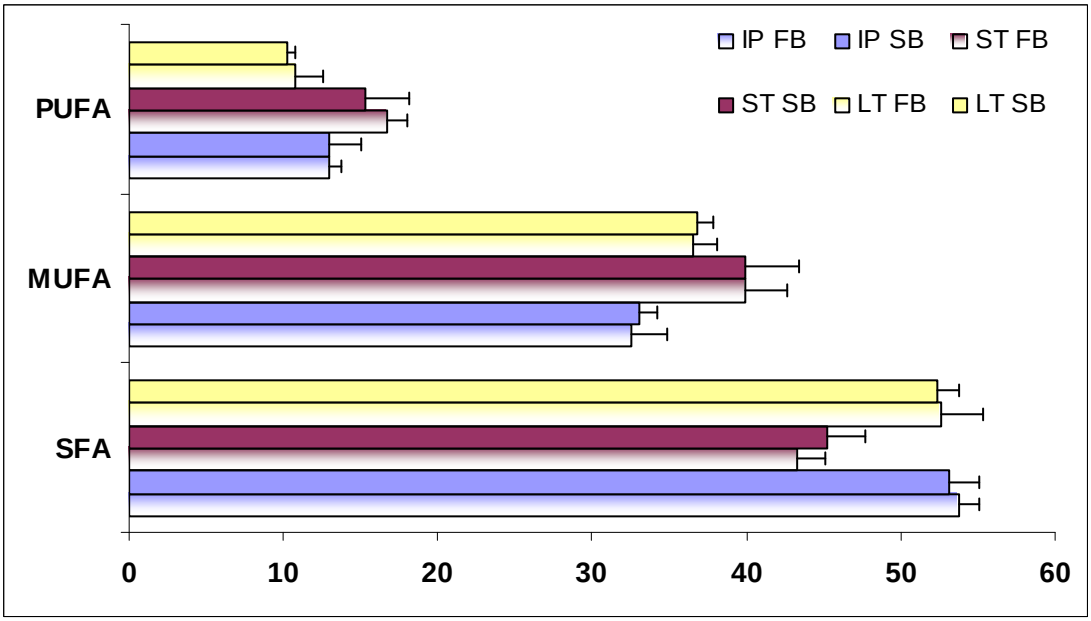
Longissimus thoracis (LT), *Semitendinosus* (ST) and *Semimembranosus* (SM)

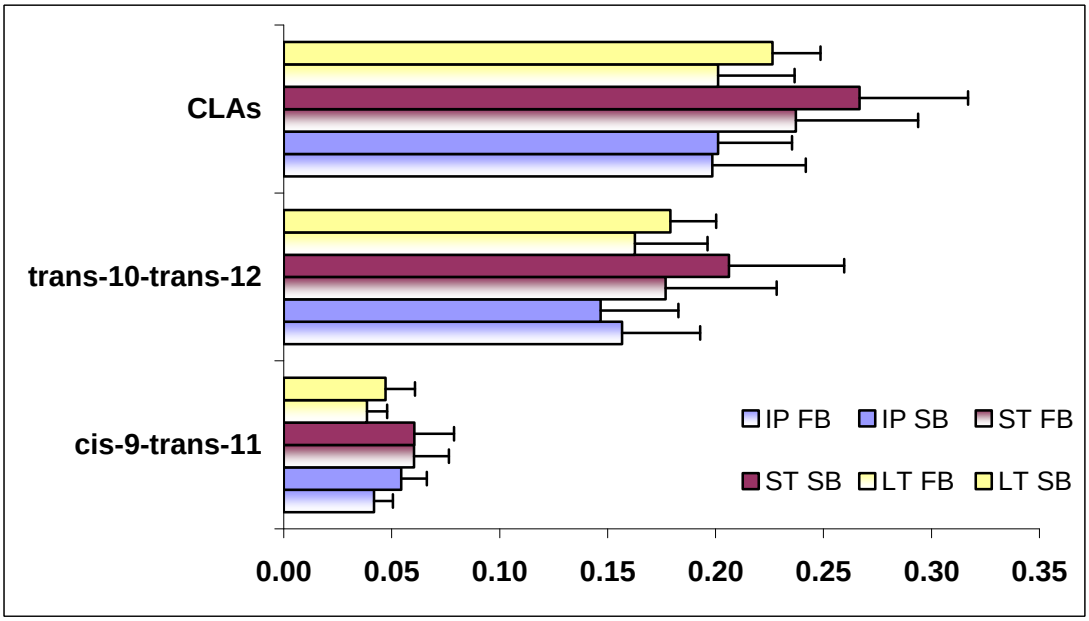
Figure 12.
Chemical composition (%): interactions between muscle and diet



FB: faba bean; SB: soya bean.
IP: *Iliopsoas* plus *Psoas minor*; ST: *Semitendinosus*; LT: *Longissimus thoracis*.

Figure 13.
Some fatty acids considered of particular nutritional significance: interactions between muscle and diet

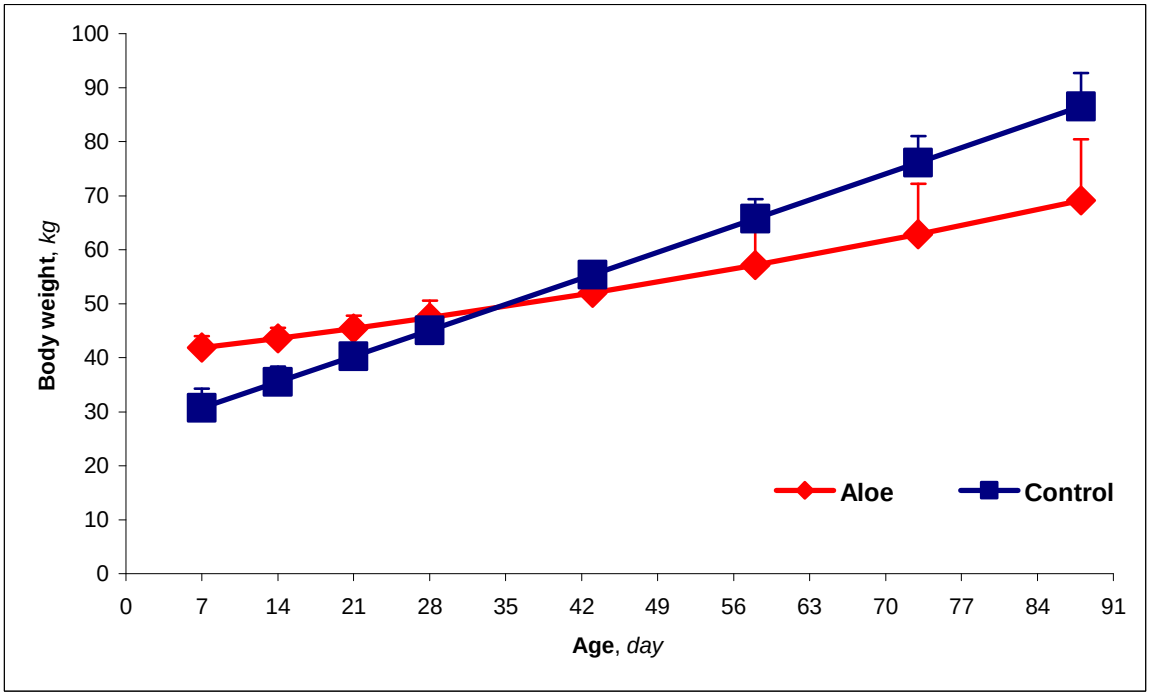




FB: faba bean; SB: soya bean.
IP: *Iliopsoas* plus *Psoas minor*; ST: *Semitendinosus*; LT: *Longissimus thoracis*.
PUFA: polyunsaturated fatty acids
MUFA: monounsaturated fatty acids
SFA: saturated fatty acids

Figure 14.

Body weight in the buffalo of two groups



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6. LIST OF ABBREVIATIONS

AI	atherogenic index
BEG	biological efficiency of growth
BW	body weight
CLA	conjugated linoleic acids
CP	crude protein
DM	dry matter
DWG	daily weight gain
FAMEs	fatty acids methyl esters
FB	faba bean
FCI	feed conversion index
IP	*Iliopsoas* plus *Psoas minor muscles*
LDL	low density lipoprotein
LT	*Longissimus thoracis* muscle
LW	live weight
MFU	milk forage units
MUFA	monounsaturated fatty acids
NPN	non-protein nitrogen
NSC	non structural carbohydrates
PUFA	polyunsaturated fatty acids
SB	soya bean meal
SFA	short-chain (C7-C11) saturated fatty acids
SM	*Semimembranosus* muscle
ST	*Semitendinosus* muscle
TI	thrombogenic index
UFV	unit forage for meat production

7. SUMMARY

The general aim of the PhD thesis, realised at the Department of Animal Science and Food Control (University of Napoli, Federico II, Italy), was to study animal performance and the nutritional characteristics of meat from Buffalo bred in Italy (*Italian Mediterranean Buffalo*) fed different diets. The importance of this research is to give a contribute to better characterize the buffalo *infra vitam* performance (i.e. weight gain, feed conversion index, etc.) and meat quality (mainly in terms of fatty acids profile). Moreover, the originality of the investigation into the field was the diet composition.

In particular, in the first experimental study the use of a leguminosae (faba bean, *Vicia faba minor* L.), as protein source in the diet of buffalo, was compared to the soya bean meal. The second investigation refers the results obtained using a nutraceutical additive (*Aloe arborescens*) in the dairy cow buffalo diets to study the influence on the colostrums quality and buffalo veal performance in the early period of life.

In the first trial it has been demonstrated that feeding growing buffalo with diet without soybean does not affect either *in vivo* performances (body weight, daily weight gain and biological efficiency of growth, feed conversion index) or nutritional characteristics of meat. This is very interesting, due to the public concerns about genetically modified feed, as large part of soybean nowadays is. The second trial confirmed the influence of good quality colostrums on the calves growth. Indeed, the addition of *Aloe arborescens* to the mother's diet during the last period of pregnancy, significantly increased the immunoglobulin content of colostrums. This result, in addition to determine and adequate passive transfer of immunity, very probably also determined the higher growing performances of calves due to the link of IgG to a number of growth and maturation factors able to increase absorption of nutrients from intestine.

Particularly interesting are the results on the fatty acids profile and cholesterol content of buffalo meat. The cholesterol content results lower than those found for the Italian bovine bred specialised for meat production; the contents of myristic, palmitic and stearic acids result significantly lower than those recorded by other authors for young

bulls of Chianina bred. To the light of the both high atherogenic and thrombogenic activity of the first two fatty acids and of that only thrombogenic of the stearic acid, we can attribute a favourable judgment to buffalo meat from the dietetic-nutritional point of view. In fact, although the contents of oleic acid and of PUFA of the ω-6 and ω-3 series are not particularly high, both the atherogenicity (AI) and the thrombogenicity (TI) index were very low.

Keywords: *Vicia faba minor*, *Aloe arborescens*, *in vivo* performance, fatty acid profile,

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