UNIVERSITA' DEGLI STUDI DI MILANO
FACOLTA' DI AGRARIA
Via Giovanni Celoria, 2 – 20133 Milano

Ph. D. (Doctorate) in Agrarian Ecology (XX Cycle)
Candidate: Dr. Damiano Magistrelli
Mat. # R05932

Coordinator: Prof. Pierluigi GENEVINI
Department of Vegetal Productions
University of Milan – Via G. Celoria, 2

Prof. Graziano ZOCCHI
Department of Vegetal Productions
University of Milan – Via G. Celoria, 2

Tutor: Prof. Fabia ROSI
Department of Animal Science
University of Milan – Via G. Celoria, 2

Didactic-Scientific Sector of the proposed work: VET/02 (Italian Ministry of Education, University and Research)

INDEX

Welfare in Goat Kids During the Peri-weaning Period: Endocrine/Metabolic Asset and Functional Development

SUMMARY

Animal welfare is the viewpoint that animals should not suffer unnecessarily, including when the animals are used for food, work, companionship, or research. The welfare of an animal is clearly affected by both failure to cope and difficulty in coping with the environment. Welfare occurs when all those factors that may reduce the ability of an animal to adapt to the environment are avoided. Those factors are named stressors and can be analyzed. An individual is stressed when control systems are overtaxed and there is an actual or potential reduction in fitness.

Stress can affect the endocrine/metabolic asset, nutritional status, normal growth and development, productivity and even the environmental impact of farm animals.

In the life of young ruminants, the most dramatic event is weaning, which often coincides with a period of growth stasis. Weaning is the process of switching young animals from milk to solid feed. This change needs adaptation in digestive activity and in rumen functions.

In light of this, the aim of the present study was to evaluate welfare status of goat kids, during the peri-weaning period, with a view to obtaining information that may help to minimize the stress of this crucial period.

For this purpose, 11 Saanen goat kids were separated from their mothers, immediately after birth, and randomly assigned to one of two groups: MILK (6 animals) and WMIX (5 animals). All kids were fed colostrum for the first three days of life. The MILK group then received *ad libitum* goat milk for the rest of the study period (to age 50 days). The WMIX group received *ad libitum* goat milk to age 29 days and then underwent weaning in which milk was progressively replaced by solid feed. WMIX kids were completely weaned on day 48 of age.

During the experimental period, the total consumption per group was recorded daily. Individual body weights were recorded weekly. Every day, all the kids were observed during expectation, administration and consumption of feeds, in order to observe eventual abnormal behaviours. Samples of the fed milk and weaning mixture were collected and analyzed for dry matter and macro-nutrients composition.

At 23, 30, 37, 44 and 50 days of age, jugular vein blood samples were taken from each animal before the first meal of the day. Plasma was analyzed for metabolic traits, such as glucose, total protein, albumin, free amino acids, urea and creatinine; for metabolic hormones, such as leptin, insulin, IGF-1 and ghrelin; and for enzymatic activity of alanine aminotransferase (ALT), aspartate

aminotransferase (AST) and α-amylase. Haematic parameters of welfare, such as cortisol and haptoglobin were determined.

At age 50 days all animals were slaughtered. Liver and carcass weights were recorded and samples of liver and pancreas were taken. Liver samples were analyzed for DNA, RNA, phospholipids and soluble protein content, ALT and AST activity. Pancreas was analyzed for DNA, RNA and zymogen content and for α-amylase activity. Data were evaluated by the two-ways analysis of variance.

During the entire experimental period, no abnormal behaviour has been recorded.

As long as all the kids were fed milk only (from day 3 to day 29), dry matter intake (DMI) was similar in both groups. Seven days after the addition of solid feed (day 36), DMI in the WMIX group began to decrease compared to the MILK group. From day 37 of age, DMI began to be significantly different between the experimental groups. The initial decrease could be due to refusal of the new diet by the WMIX animals. Subsequently, lower DMI in this group could be because products from ruminal activity increasingly contributed to the nutritional requirements of the animals. Despite the difference observed in DMI, body weight did not differ between the two groups throughout the entire period.

Overall means of plasma glucose and free amino acids were lower in WMIX kids, creatinine was higher in WMIX group, compared to MILK one. The largest differences were found at age 50 days, when glucose, free amino acids and urea were significantly lower, and creatinine was significantly higher in the WMIX than the MILK group. The lower glucose and free amino acids in WMIX kids could have been due to the relative inability of the solid diet to supply sufficient protein and energy. The lower plasma urea in the WMIX group may have been a consequence of urea recycling to the rumen. The development of rumen activity may have stimulated urea recycling to the rumen for microbial protein synthesis, thereby enhancing the efficiency of protein utilization. Furthermore, since only in the MILK group plasma urea was directly related to amino acid levels, it is possible that animals of this group used dietary amino acids as energy source as well as to meet their anabolic needs. The higher levels of plasma creatinine in the WMIX group might be the consequence of an initial tissue wasting, due to the change of the diet, not yet resulting in alteration in carcass weight.

At 50 days of age, plasma insulin and IGF-1 were more than three times lower and ghrelin was significantly higher in the WMIX than the MILK group. As ghrelin acts on the central nervous system to stimulate food intake, the higher plasma ghrelin levels in the weaned animals could have been due to the lower DMI, which was particularly low at the completion of weaning. The

lower levels of insulin and IGF-1 in WMIX kids may have been related to the lower plasma glucose and amino acids in the WMIX group. In fact, at this time, both plasma insulin and IGF-1 strongly correlated to glucose and amino acids. The possibility arises that the higher insulin and IGF-1 in the MILK group could be due to insulin and IGF-1 supplied with the milk. In fact, both hormones are naturally present in goat's milk. Moreover, several milk-borne peptides can pass the gastro-intestinal mucosa in a receptor-mediated process to enter the systemic circulation and supplement the suckling animal's production of these substances.

Two days after the completion of weaning, plasma activity of ALT and AST were significantly higher in the WMIX group than the MILK one, as possible consequence of the change of the diet. In humans, in fact, the reduction of protein intake may increase plasma ALT and AST activity.

Plasma cortisol level began to be higher in the MILK group on day 44 of age. By contrast, no difference was observed in the concentration of plasma haptoglobin, during the entire study period. The correlation between plasma urea and amino acid levels in MILK kids induces to hypothesize that animals of this group used a part of the dietary amino acids as energy source. So, their higher level of plasma cortisol could have stimulated the liver to convert amino acids to glucose for energy.

No difference was observed in carcass weight between the experimental groups. This letter result, together with data obtained on cortisol and haptoglobin, suggests that the weaning process adopted in the present study was not stressful and did not affect animal welfare.

Liver glycogen was higher in the MILK kids, as possible consequence of the higher level of plasma glucose in these animals.

Finally, both pancreatic amylase activity expressed as international units (IU) per gram of fresh tissue and pancreatic amylase activity expressed as IU per mg of DNA were more than three times lower in the WMIX than the MILK group. This latter result is surprising as the presence of starch in the diet of the WMIX animals did not stimulate pancreatic α-amylase activity. Even more intriguing is the fact that the greater α-amylase activity in milk-fed kids could have been stimulated by the higher insulin levels, which were possibly increased by the consumption of milk-borne insulin. In fact, in rats there is a close relationship between the increased plasma insulin just before weaning, and pancreatic α-amylase activity, suggesting that insulin may stimulate pancreatic functional development. In support of this, plasma insulin was strongly correlated to pancreatic α-amylase.

In conclusion, the adopted weaning process minimized the stress of the transition from milk to solid diet. During this transition, milk-borne insulin played a possible role in the development of pancreatic amylase synthesis and

activity. Further studies are required to determine whether milk-borne insulin passes the gastro-intestinal mucosa of suckling kids and contributes to the functional development of the pancreas.

1 INTRODUCTION

1.1 Animal Welfare

Animal welfare is commonly considered as the viewpoint that animals should not suffer unnecessarily, including when the animals are used for food, work, companionship, or research (Medical Research Council, 1999).

Suffering occurs when unpleasant subjective feelings are acute or continue for a long time because an animal is unable to carry out the actions that would normally reduce risks to life and reproduction in those circumstances (Dawkins, 1990).

Suffering and poor welfare often occur together, but welfare is a somewhat wider term. Unpleasant subjective feelings will clearly affect the state of an individual as regards its attempts to cope with its environment. However, it could be that the state is affected without suffering occurring (Broom, 1991). In addiction to the absence of sufferings, animals have a wide range of needs that are a consequence of the many functional systems that make life possible. A need is a deficiency in an animal that can be remedied by obtaining a particular resource or responding to a particular environmental stimulus (Fraser and Broom, 1990).

If an animal has a need, its motivational state is affected so that behavioural and physiological responses that should result in remedying that need can be made. These coping responses allow the animal to control and maintain mental and bodily stability. The animal may succeed in its attempts to cope with the conditions in which it finds itself, in which case it has adapted to those conditions. Sometimes it may succeed only with great difficulty. Alternatively, it may fail to cope and the individual lacks control over interactions with its environment (Broom, 1988a).

The welfare of an animal is clearly affected by both failure to cope and difficulty in coping (Broom, 1991). In light of this, welfare may be considered as "the state of an individual in relation to its ability to adapt to the environment" (Broom and Johnson, 1993).

1.2 Animal Welfare and Ecology

The term "Ecology" was coined by the German biologist Ernst Haeckel in 1866 when he defined it as "the comprehensive science of the relationship of the organism to the environment". Haeckel did not elaborate on the concept and the first significant textbook on the subject – together with the first university course – was written by the Danish botanist Eugenius Warming (1895). For this early work, Warming is often identified as the founder of Ecology.

The word "Ecology" derives from Greek οικοζ[1] and λογοζ[2] and, today, it is considered the scientific study of the processes regulating the distribution and abundance of organisms and the interactions among them, and the study of how these organisms in turn mediate the transport and transformation of energy and matter in the biosphere (Krebs, 1972).

All the organisms that live in the environment are in a close relation with it: they constantly attempt to modify it or adapt to it with the aim to maintain and extend life, as it represents the principle of life itself.

The maintenance of life is the result of a complex bidirectional flux of materials and energy between the individual and its environment. Balance only occurs when this bidirectional flux takes place avoiding damages to all the component of the system.

In light of this, it's not possible to dissert on ecology without considering the environmental impact of farm animals. Moreover, as this impact is dependent on the state of welfare of animals, it is possible to assume that ecology is strictly concerned to animal welfare.

1.3 The concept of Stress

In accordance to its definition, welfare occurs when all those factors that may reduce the ability of an animal to adapt to the environment are avoided (Amadori et al., 2002). "Those factors" are named "stressors". An individual is stressed when control systems are overtaxed and there is an actual or potential reduction in fitness (Broom, 1988b).

The term "stress" was used for the first time by the Canadian endocrinologist of Austro-Hungarian origin Hans Hugo Bruno Selye in 1936. He defined it as a "General Adaptation Syndrome" (GAS): a typical syndrome that appears if an organism is severely damaged by acute non-specific nocuous

[1] Oikos: household

[2] Logos: knowledge

agents, the symptoms of which are independent of the nature of the damaging agent (Selye, 1936). Stress is a physiological response to environmental changes produced by external or internal factors which involves the whole organism (Selye, 1951).

Selye discerned between two different kinds of stress: "distress", having negative implications, and "eustress", considered a positive form of stress, usually related to desirable events or producing good reactions. Both can be equally taxing and both are cumulative (Selye, 1975). Distress is the most-commonly referred to type of stress.

1.4 Endocrine/Metabolic Effects of Stress

In his seminal work on the general adaptation syndrome, Selye observed marked changes in the sizes of endocrine tissues subsequent to stressors exposure (Selye, 1939). Selye's observations have been confirmed many times over, and it is clear that endocrine responses constitute an integral component of the stress response (Van de Kar et al., 1991; Stratakis and Chrousos, 1995). Stress can affect the hormonal control of metabolism (Weissman, 1990; Wenk, 1981), reproduction (Moberg, 1991; Rivier and Rivest, 1991), growth (Stratakis et al., 1995) and immunity (Peterson et al., 1991; Sheridan et al., 1998). Since hormone signalling plays a vital role in the maintenance of homeostasis, virtually every endocrine system responds in some fashion to specific stressors. The overall effect on the animal's adaptive response to stress is an integration of multiple, and often interactive, hormone responses that directly affect physical health and well-being (Matteri et al., 2000).

One of the best known and consistent neuroendocrine responses to stress is the activation of the hypothalamus-pituitary-adrenal axis (HPA), resulting in the secretion of steroid hormones from the adrenal gland (Matteri et al., 2000). This relationship between stress and adrenocortical activation was one of the first recognized in the study of the endocrinology of stress (Selye, 1939). The regulation of glucocorticoids secretion from the adrenal gland depends on a linkage between the hypothalamus and the pituitary gland: factors produced in hypothalamic neurons regulate the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Guillemin and Rosenberg, 1955; Saffran et al., 1955; Porter and Jones, 1956). ACTH stimulates the synthesis and release of steroids from the adrenal cortex by promoting the uptake of cholesterol and its enzymatic conversion to cortisol and corticosterone, the glucocorticoid hormones. Glucocorticoids play an important role in gluconeogenesis by

stimulating the liver to convert fat and protein to intermediate metabolites that are ultimately converted to glucose for energy (Matteri et al., 2000).

Glucocorticoids also support this response by potentiation of the synthesis and action of epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine: catecholamines that influence the HPA axis at many levels, including stimulating neurohormone release from the hypothalamus (Plotsky et al., 1989), ACTH release from the pituitary gland (Giguere and Labrie, 1983; Axelrod and Reisine, 1984; Dinan, 1996) and cortisol release from the adrenal cortex (Dinan, 1996).

The maintenance of a sufficient, yet not excessive, concentration of glucocorticoids is necessary in order to maintain homeostasis. Chronic elevation of glucocorticoids results in protein catabolism, hyperglycaemia, immune suppression, susceptibility to infection and depression (Matteri et al., 2000).

Chronic elevation of corticosteroids can also affect mental performance and reduce hippocampal volume (McEwen and Sapolsky, 1995).

Considering the potent deleterious actions of chronically elevated glucocorticoids, an important function of these steroids is to curtail the HPA response to stress. This occurs through negative feedback inhibition, where glucocorticoids inhibit further HPA response at the levels of the brain and pituitary (McEwen, 1979; Fink et al., 1991).

Factors involved in the regulation of ACTH secretion are corticotropin-releasing hormone (CRH) and vasopressin (VP), which work independently, as well as in concert, to mediate glucocorticoids secretion at the level of the pituitary and adrenal glands. CRH and VP may also regulate glucocorticoids production and secretion via paracrine (local) actions within the adrenal gland. The magnitude and duration of the glucocorticoid response are secretogogue-dependent (Matteri et al., 2000).

Another regulatory factor that has been reported to induce ACTH secretion from the anterior pituitary is oxytocin (Link et al., 1993). In fact, high affinity receptors for oxytocin have been identified in the rat pituitary gland (Antoni, 1986). The biological need for multiple stimulators of ACTH secretion has not been fully elucidated; however, the existence of multiple stimulators does highlight the relative importance of this system in the maintenance of homeostasis.

Some authors suggest that specific stressors elicit specific patterns of neuro-hormonal activation, supporting the concept that activation of the HPA axis is stressor specific (Mason, 1974; Seggie and Brown, 1982). The effectiveness of glucocorticoid-mediated negative feedback on the HPA axis also varies among specific stressors (Plotsky et al., 1993). It would appear that the brain has the ability to distinguish between stressors and to release ACTH

secretogogues depending on the physiological response needed to cope with the current stressor. Similarly, plasma concentrations of catecholamines, which regulate the HPA response, are elevated by acute stress, but the magnitude of the increase is dependent on the intensity of the stressors (Natelson et al., 1981; Goldstein et al., 1983).

Stress may also affect growth hormone (GH) secretion by the anterior pituitary gland and insulin-like growth factor 1 (IGF-I) secretion by the liver. IGF-1 is a 7.6 kDa peptide which is considered to have a crucial role in mediating the effect of GH in the regulation of growth and differentiation on a variety of peripheral tissues, during post-natal life (Breier, 1999). The anabolic action of plasma IGF-1 consists in stimulating the uptake of amino acids and glucose by the cells (Noguchi, 2000). Stress-induced reductions of GH and IGF-I secretion has been reported in rats (Armario et al., 1987; Straus, 1994; Peisen et al., 1995); whereas data from other vertebrate species indicate that the somatotrophic axis responds to stress by concurrently increasing GH and decreasing IGF-I secretion (Vance et al., 1992; Kakizawa et al., 1995; Bruggeman et al., 1997; Carroll et al., 1998; McCusker, 1998). The increase in circulating GH antagonizes the effects of insulin by direct GH receptor-mediated actions on peripheral target tissues, thus reserving blood glucose. A reduction in IGF-I is thought to minimize growth during times of distress, further preserving energy. Concurrent elevation in GH and suppression of IGF-I secretion in non-rodent species seem to be an important adaptive response that diverts energetic substrates from growth to survival (Matteri et al., 2000). GH response can be enhanced by glucocorticoids (Casanueva et al., 1990). By contrast, a rapid reduction in IGF-I level can be induced by cortisol (McCarthy et al., 1990).

Most pituitary and hypothalamic hormones can be influenced by stress. Acute psychological stress elevates PRL secretion in a variety of species (Klemcke et al., 1987; Jurcovicova et al., 1990; Kirschbaum et al., 1993; Matthews and Parrott, 1994; Juszczak, 1998).

The best-documented function of PRL is to stimulate milk synthesis and secretion; however, a variety of other functions may exist (Bole-Feysot et al., 1998). PRL seems to play a role in the maintenance of the corpus luteum (Bazer et al., 1991) and it is implicated in the control of salt-water balance, immunity, growth, development and metabolism (Nicoll, 1980; Weigent, 1996). Circulating level of PRL arises in response to stress (Matteri et al., 2000). Since there are several potential functions of PRL in vertebrates, it's difficult to assign a specific adaptive purpose to stress-induced PRL secretion. There is speculation, however, that PRL may induce analgesia and offer protection against stress effects (Drago et al., 1989). While most of the PRL responses to stress are characterized by increase in hormone secretion, reduced

PRL level may occur as a consequence of chronic-stress, such as that encountered during prolonged illness (Van den Berghe and de Zegher, 1996).

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are collectively termed gonadotropins due to their positive effects on gonadal structure and function. These hormones are produced in specialized cells of the anterior pituitary gland called gonadotrophs. Acute stress can elicit a short-lived increase in LH, but not FSH, secretion (Siegel et al., 1981; Sakamoto et al., 1991), whereas chronic stress is recognized as a cause of decreased gonadotropin secretion and reproductive failure (Moberg, 1991; Rivier and Rivest, 1991). In cases of emergency, the pituitary tends to produce more adrenocorticotropic and less gonadotropic hormone than under normal conditions. The reason for this is probably that, under certain conditions, an abundant supply of the life-maintaining principle of the adrenal cortex is a more imminent necessity than the preservation of normal sex function' (Selye, 1939). The neural and endocrine mechanisms underlying stress-induced reproductive failure are complex and have yet to be fully elucidated, but scientific evidences exist for suppressive effects of glucocorticoids, vasopressin, ACTH and CRH on gonadotropin secretion (Matteri et al., 1984; Moberg, 1991; Ferin, 1993; Rivest and Rivier, 1995; Dobson and Smith, 1995; Xiao et al., 1996).

Stress can affect hypothalamic thyrotropin-releasing hormone (TRH), pituitary thyroid-stimulating hormone (TSH) and the thyroid hormones tri- and tetra-iodothyronine, (T3 and T4).

TRH is released into the hypophysial portal vessels, where it is carried to the anterior pituitary. The pituitary cell type that produces and secretes TSH in response to TRH is called the thyrotroph. As potent metabolic regulators, thyroid hormones play a major role in controlling body temperature and metabolism (Danforth and Burger, 1984).

The effect of acute stress on TSH secretion may vary between species. Short-term stress produces a transient increase in TSH secretion in humans (Richter et al., 1996), whereas a decrease in secretion is observed in rodents (Goya et al., 1995; Marti et al., 1996).

The physiological benefit of stress-altered TSH secretion has not been elucidated yet (Matteri et al., 2000).

1.5 Influence of Stress on Appetite

The suppression of appetite due to stress has long been discussed. Various stressors may influence neural and neuroendocrine mechanisms involved with appetite control (Forbes, 1995).

Regulation of food intake is currently a topic of great interest in human and animal science.

Regions of the hypothalamus have long been associated with the control of essential homeostatic functions including appetite.

The most potent stimulators of feed intake in animals are neuropeptide Y (NPY) and Aguti-related peptide (AgRP). NPY has a 36 amino acids structure (Tatemoto et al., 1982) whereas AgRP is composed by 132 amino acids (Wilczynski et al., 2004). NPY and AgRP are co-expressed in arcuate nucleus neurons of the hypothalamus. NPY and AgRP are traditionally classified as food-stimulating neuropeptides and feed restriction increases both transcription and levels in the hypothalamus, indicating that these highly co-localized neuropeptides are also highly co-regulated following fasting (Kas et al., 2005). The main opponent to NPY/AgRP synthesis is leptin (Houseknecht and Portocarrero, 1998; Kas et al., 2005). Leptin is a 16 kDa protein produced by the *ob* gene (Zhang et al., 1994). Leptin is expressed in many tissues, including skeletal muscle, gastric epithelium, brain, placenta (during pregnancy) and mammary gland (during lactation) (Ahima and Flier, 2000). Anyway, the main site of leptin secretion is white adipose tissue (Auwerz and Staels, 1998). Leptin is a 167 amino acid peptide with an amino-terminal secretory signal sequence of 21 amino acids. The translocation of leptin into microsomas of adipose cells is followed by the subsequent removal of the signal peptide and secretion into the blood stream. Circulating leptin is a peptide of 146 amino acids with a molecular mass of 14 kDa (Prolo et al., 1998).

Adipocytes produce and secrete leptin in quantities directly and positively correlated with the adiposity of the animal and thus indirectly correlated with body weight. Leptin receptors have been identify within the arcuate (ARC, lower medial) area of the hypothalamus of sheep (Dyer et al., 1997) and they are co-localized with NPY/AgRP neurons (Mercer et al., 1996).

In normal animals, leptin and NPY/AgRP work in concert to maintain energy balance. Feedback mechanisms exist which would further ensure homeostasis. Leptin receptors appear to be down-regulated by leptin and highly expressed when leptin level is low (Dyer et al., 1997; Mercer et al., 1997; Baskin et al., 1998).

A variety of other peptides may influence appetite.

Insulin is a 5.8 kDa protein synthesized in the pancreatic ß-cells and secreted in response of elevation of plasma glucose level. Insulin is responsible

for glucose uptake from the blood stream into adipose, muscular and hepatic cells with a mechanism similar, although much quicker, to that of IGF-1 (Breier, 1999; Pessin and Saltiel, 2000), but it also modulates peripheral satiety signals and directly targets the central nervous system to inhibit food intake. Insulin and leptin share many properties: in a manner similar to leptin, insulin crosses the blood-brain barrier and interacts with specific receptors in the arcuate nucleus of the hypothalamus reducing food intake and body weight in a dose-dependent manner (Gale et al., 2004).

By contrast, ghrelin is a 28 amino-acids acylated peptide from stomach, which plays a role in stimulating GH release by the pituitary gland and in affecting feeding behaviour as stimulator of food intake. Ghrelin-containing neurons have been identified in the arcuate nucleus of the hypothalamus, where directly enforce the release of NPY/AgRP, potently stimulating food intake (Kojima and Kangawa, 2005).

The recently discovered neuropeptide orexin (ORX) also increases feed intake in adult rats (Sakurai et al., 1998) and in weanling pigs (Dyer et al., 1999). Like NPY/AgRP, orexin mRNA expression increases in rats which have been feed-restricted (Sakurai et al., 1998).

Stressors differently affect the level of pepdides involved in the control of appetite. In example, glucocorticoids secretion and inflammatory stress increase serum leptin level (Mastronardi et al., 2001), but hypothermia consistently decreases leptin expression (Trayhurn et al., 1995). Endogenous-induced acute stress results in a rapid decrease in plasma leptin (Schafroth et al., 2001), whereas there is no clear effect of surgical stress on the release of leptin into the circulation (Mastronardi et al., 2001). In human and rats, glucocorticoids increase plasma leptin level. This suggests that the decrease of feed intake associated with stress is at least partially induced by the increase in leptin secretion (Newcomer, 1998). By contrast, a recent report states that ghrelin augments ACTH release in response to stress secretion-inhibiting the effect of leptin (Ueta et al., 2003). Anyway, the roles of leptin and ghrelin in stress responsiveness in farm animals needs further investigations.

Insulin level arises in consequence of stress, to contrast the arise of glucose caused by glucocorticoid secretion. Anyway, cases of insulin-resistance are usually observed during stressor exposure (Burén and Eriksson, 2005; Chan et al., 2005). This is probably due to the insulin-antagonistic action of hormones like glucocorticoids. Insulin resistance may develop diabetes (Burén and Eriksson, 2005).

Data reported on ghrelin inhibiting effect on leptin and insulin-resistance suggest that feed intake may increment during stressors exposure. Moreover, acute stress generally increases neuropeptide Y mRNA within the arcuate nucleus of hypothalamus (Conrad and McEwen, 2000). However, NPY up-

regulation within the arcuate nucleus does not reflect a general up-regulation of brain NPY gene expression. It happens because NPY and AgRP mRNA expression in the arcuate nucleus are dissociated after a stressful event. (Kas et al., 2005), thus explaining the stress-induced suppression of feed intake in farm animals.

1.6 Nutritional Stress

Nutritional stress poses a serious challenge to homeostasis. The importance of nutritional intake in maintaining reproductive function is well established (Chilliard et al., 1998). Inadequate nutrition delays or prevents the onset of puberty, interferes with normal cyclicity in the female and results in hypogonadism and infertility in males (Matteri et al., 2000).

A consistent observation across species is that undernutrition results in decreased gonadotropin secretion (Schillo, 1992; Brown, 1994). A complex array of neural and neuroendocrine signals convey information of nutritional status to the reproductive system. More recent evidence suggests that leptin is required for reproduction (Houseknecht et al., 1998; Vitali et al., 2005). Reduced leptin secretion due to inadequate nutrition may play an important role in mediating the associated disruption of reproductive neuroendocrine function (Houseknecht et al., 1998; Vitali et al., 2005). Analogously, stress-induced interferences to leptin secretion and biological activity must be avoided in order to ensure normal reproduction.

Nutritional stress also decreases overall activity of the thyroid axis. The elevation in HPA activity during chronic undernutrition may contribute to suppressed activity of the thyroid axis. Infusion of cortisol at level similar to that observed during fasting suppresses TSH secretion (Samuels and McDaniel, 1997). In addition to reducing thyrotroph function, undernutrition diminishes hypothalamic TRH release, thyroid hormone production and levels of peripheral thyroid hormone receptors (Diano et al., 1998; Matteri et al., 2000). The decrease in thyroid axis function at so many levels reflects an important adaptive response to undernutrition. A reduction in metabolic rate and associated energy use has a positive survival value at times when the food supply is limited (Chilliard et al., 1998).

1.7 Immune System Response to Stress

Organisms have evolved many strategies to defend against stressful environments and conditions. Indeed one could argue that the immune system is the result of the evolving response of the host to the stress of pathogen challenge. Exactly how the immune system responds to stress, however, is a difficult question to answer, in part because of the complexities of the host immunity and the stress response systems (Blecha, 2000).

The assumption that stress influences host immunity arises from observations of increased disease occurrence in animals exposed to extreme stressful environments (Blecha, 2000).

Calves exposed to stressful conditions develope a leukocytosis that is caused primarily by a neutrophilia. Neutrophilia is a common observation in stressed animals and is thought to be caused by a glucocorticoid-induced change in neutrophil trafficking and release from bone marrow reserves (Kelley et al., 1981; Roth and Kaeberle, 1982; Murata et al., 1987).

Together with evidences on the stress-induced increase in cortisol concentrations and neutrophilia, there are also studies that have failed to show a direct correlation between concentrations of plasma cortisol induced by various stressors and immune function, suggesting that other factors, in addition to cortisol, are involved in stress-induced immune alterations (Blecha, 2000).

Although acute increase in glucocorticoids is considered a classic characteristic of the stress response – as indicated previously – it is often difficult to establish a relationship between stress-induced activation of the HPA axis and alterations in immune function (Blecha, 2000).

Anyway, it has been observed that some stressful conditions (such as physical restraint) can increase ACTH and cortisol concentrations in pigs and lambs, together with alteration of some immune parameters, including lymphocyte proliferative responses and expression of some leukocyte differentiation antigens (McGlone et al., 1993; Minton et al., 1992).

1.8 Effect of Stress on Productivity

Farm animal agriculture has experienced massive change since the turn of the century. Mechanization and the implementation of new technologies have resulted in major increases in efficiency and intensity of production (Swanson, 1995).

Intensive farming practices have become common because of the rapid increase in the global human population. The old style of agriculture and

livestock can no longer produce enough food to feed all the people in the world. However, not always "more" means "better" (Barton, 2002). In fact, excessively intense livestock practice may lead to a stressor situation in which animals are no longer able to maintain homeostasis and can develop ipofertility and metabolic diseases and reduce resistance to pathogens, compromising the productivity itself of the farm (Verga, 2000).

Many intensive livestock production management practices may affect host immunity and disease susceptibility (Blecha, 2000). One of the most frequently used examples of this relationship is bovine respiratory disease in cattle. The bovine respiratory disease complex has been studied intensely for decades, yet it continues to cause large economic losses in the cattle industry. This multifaceted disease complex involves the interaction of respiratory viruses and bacteria and stress (Loan, 1984).

In addition, a reduction in body weight has been observed in stressed pigs compared to unstressed ones (McGlone et al., 1993), thus confirming the possibility for stress to affect farm productivity.

For this reason, in the last years, together with intensive farming systems, farmers are trying to develop new technologies able to improve livestock quality and animal well-being (Verga and Ferrante, 2002), in order to increase the quality of the end products (Carenzi et al., 2001).

1.9 Stress and Environmental Impact

As reported above, physical or psychological stress affects various physiological factors, such as the secretion of hormones, the activity of autonomic neurons, and the regulation of the immune system. These factors are closely associated with the regulation of gastrointestinal functions. Both in humans and experimental animals, many reports have indicated that stress affects gastric emptying, gastric secretion, intestinal transit, and colonic motility (Williams et al., 1988; Plourde, 1999). In the colon, stress has been reported to stimulate motility via the activation of autonomic neurons, increasing faecal pellet output, or diarrhoea (Okano et al., 2005). The mechanism of this increase has not been fully elucidated, but it is usually associated to a significant increase in the plasma concentration of ACTH. In gerbils under novelty stress, faecal pellet output increases 1.5 times, while ACTH level is quadruple, in relation to non-stressed animals (Okano et al., 2005).

The stimulation of intestinal motility and the increase in faecal output under stressful conditions may indicate that stress can reduce the efficiency of

dietary nutrients utilization. Moreover, the latter data represent evidences that stress can affect the environmental impact of animals and enforce the theory that ecology is strictly concerned to animal welfare.

1.10 The Five Freedoms

The Five Freedoms are a well-established set of propositions which provide a core framework encompassing an animal's basic needs, whether on farm, in transit, at market or at the place of slaughter. They declare a series of "freedoms" and implied husbandry requirements, which underlie good farm animal welfare, involving:

1. Freedom from hunger and thirst, by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from discomfort, by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury and desease, by prevention or rapid diagnosis and treatment.
4. Freedom to express normal behaviour, by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from fear, by ensuring conditions and treatment which avoid mental suffering.

The concept of the "five freedoms" appeared for the first time in 1965, in the "Report of the Technical Committee to Enquire Into the Welfare of Animals Kept Under Intensive Livestock Husbandry System" of the British Government. That concept was precisely defined in April 1999, when the Farm Animal Welfare Council (FAWC) was asked by the English Ministry of Agriculture, Fisheries and Food (MAFF) to consider the relationship between farm assurance schemes and animal welfare.

The content of the "five freedoms" have guided FAWC's philosophy of approach in all its studies. They provide widely accepted guidelines to all concerned with the keeping of livestock as to how they might fulfil their obligation to the animals they use at every stage of production. The freedoms have
provided the backdrop to the most of the studies into the relationship between farm assurance schemes and farm animal welfare (FAWC, 2001).

1.11 Indicators of Stressful Conditions

Farm animal welfare is usually measured using different indicators (Pol et al., 2002). Although the most of the present treatment focuses on the effects of stress on metabolism and immune system, one of the best known method to provide information on animal welfare condition is animal observation (Pol et al., 2002).

The FAWC's fourth freedom states that animals should be able to express their normal behaviour patterns. One interpretation of this freedom is that, in general, animals that can perform normal behaviour are more likely to achieve better welfare than those that cannot (Kilgour, 1978). According to this, abnormal behaviours can be considered as indicators of poor welfare (Gonyou, 1994). However, what are "abnormal behaviours"? Some examples of these behaviors are aggression, injurious behaviors such as feather pecking or tail biting, and repeated movements, or stereotypies (Gonyou, 1994). Unfortunately, many behaviours that are considered indicative of poor welfare have multiple causes, so stress assessment requires more specific evaluation methods (Pol et al., 2002).

Stress is usually assessed by the level of plasma cortisol, which reflects the activity of the HPA axis (Brown-Borg et al., 1993; Bradshaw et al., 1996; Pol et al., 2002). However, plasma levels of cortisol vary with circadian rhythm, season and photoperiod or food intake rhythm (Barnett et al., 1981). Moreover, blood sampling itself causes a rise in cortisol level, so the withdrawal must be done as carefully and as rapidly as possible, in order to avoid restrain stress (Magnusson et al., 1998).

In light of this, together with cortisol, metabolic and endocrine traits must be determined, in order to evaluate correctly the effect of stressful conditions on the whole body homeostasis. In particular, as stress may induce the suppression of feed intake, the analysis of hormones involved in the regulation of feeding behaviour (such as leptin, insulin and ghrelin) can be a useful tool to evaluate the presence of stressful conditions.

Another haematological parameter for the evaluation of the welfare status is represented by a group of plasmatic proteins called Acute Phase Proteins (APP). The most investigated APP is haptoglobin. APP are synthesized by the liver and their level rapidly increase in respond to pathogens infections and stressful events (Asai et al., 1999; Chen et al., 2003).

1.12 Stress and Weaning

In the life of farm animals, it is often possible to attend to a period of growth stasis, witch coincides to weaning (McCracken et al., 1995).

Weaning is the process of switching young animals from milk to solid feed. Usually, in ruminants weaning is not just an event, but a period in which milk is progressively substituted by forage and concentrate or grain-based diets. The weaning program is generally chosen according to economic and practical criteria, since it can influence growth (Owens et al., 1993), maturation of the reproductive tract (Lawrence et al., 2005) and even the quality of the end products (Schoonmaker et al., 2002).

During the weaning period, diet shifts from a mixture of casein, lactose and triglyceride to a more complex source of nutrients. This change needs adaptation in rumen functions (Baldwin et al. 2004) and digestive activity (Kinouchi et al. 2000); in example, administration of vegetable feeds, containing starch, requires the secretion of novel digestive enzymes in the small intestine, even if a part of it could be fermented in the developing rumen (Ortega-Cerrilla and Mendoza-Martinez, 2003).

Weaning-induced stress could be the result of the interaction between new dietary substrates and developing ruminal activity. Weaning is a dramatic metabolic event for young mammals, in whom the maturation of the digestive system is still incomplete (Kelly and Coutts, 2000).

In light of this, it is worth of interest to investigate the effects of weaning, with a view to obtaining information that may help to minimize stress, improving animal welfare and productivity and reducing the environmental impact of farm animals, during this crucial period.

1.13 Literature Cited

Ahima R. S. and Flier J. S.: Leptin. Annual Review of Physiology, 2000; 62: 413-437.

Amadori M, Archetti I. L., Mondelli M. M. and Fazia M.: La Valutazione del Benessere Animale. In: La Valutazione del Benessere nella Specie Bovina. Edited by Amadori M. and Archetti I. L. Published by Fondazione Iniziative Zooprofilattiche e Zootecniche - Brescia, Italy; May 2002, pp 13-21.

Antoni F.A.: Oxytocin Receptors in Rat Adenohypophysis: Evidence From Radioligand Binding Studies. Endocrinology, 1986; 119: 2393-2395.

Armario A., Garcia-Marquez C. and Jolin T.: Crowding-induced Changes in Basal and Stress Levels of Thyrotropin and Somatotropin in Male Rats. Behavioral and Neural Biology, 1987; 48: 334-343.

Asai T., Mori M., Okada M., Uruno K., Yazawa S. and Shibata I.: Elevated Serum Haptoglobin in Pigs Infected With Porcine Reproductive and Respiratory Sindrome Virus. Veterinary Immunology and Immunopathology, 1999; 70:143-148.

Auwerx J. and Staels B.: Leptin. The Lancet, 1998; 351(3): 737-742.

Axelrod J. and Reisine T. D.: Stress Hormones: Their Interaction and Regulation. Science, 1984; 224: 452459.

Baldwin R. L., McLeod K. R., Klotz J. L. and Heitmann R. N.: Rumen Development, Intestinal Growth and Hepatic Metabolism in the Pre- and Post-weaning Ruminant. Journal of Dairy Science, 2004; 87 (E. Suppl.): E55-E65.

Barnett J. L.,Winfield C. G., Cronin G. M. and Makin A. W.: Effects of Photoperiod and Feeding in Plasma Corticosteroid Concentrations and Maximum Corticosteroid Binding Capacity in Pigs. Australian Journal of Biological Science, 1981; 34: 577-585.

Barton F.: Animal Productivity and Animal Welfare: Letter to the Editor. Canadian Veterinary Journal, 2002; 43: 228.

Baskin D. G., Seeley R. J., Kuijper J. L., Lok S., Weigle, D. S., Erickson J. C., Palmiter R. D. and Schwartz M.W.: Increased Expression of mRNA for the Long Form of the Leptin Receptor in the Hypothalamus is Associated with Leptin Hypersensitivity and Fasting. Diabetes, 1998; 47: 538-543.

Bazer F. W., Simmen R. C. and Simmen F. A.: Comparative Aspects of Conceptus Signals for Maternal Recognition of Pregnancy. Annals of the New York Academy of Sciences, 1991; 622: 202-211 .

Blecha F.: Immune System Response to Stress. In: The Biology of Animal Stress. Edited by Moberg G. P. and Mench J. A. Published by CAB International, 2000, pp. 111-121.

Bole-Feysot C., Goffin V., Edery M., Binart N. and Kelly P. A.: Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways and Phenotypes Observed in PRL Receptor Knockout Mice. Endocrine Reviews, 1998; 19: 225-268.

Bradshaw R. H., Parrott R. F., Goode J. A., Lloyd D.M., Rodway R. G. and Broom D. M.: Behavioural and Hormonal Responses of Pigs During Transport: Effect of Mixing and Duration of Journey. Animal Science, 1996; 62: 547-554.

Breier B. H.: Regulation of Protein and Energy Metabolism by the Somatotropic Axis. Domestic Animal Endocrinology, 1999; 17: 209-218.

Broom D. M.: The Scientific Assessment of Animal Welfare. Applied Animal Behavioural Science, 1988a; 20: 5-19.

Broom, D. M.: Les Concepts de Stress et de Bien-être. Recherche de Medicine Veterinarie, 1988b; 164: 715.

Broom D. M.: Animal Welfare: Concepts and Measurement. Journal of Animal Science, 1991; 69: 4167-4175.

Broom D. M. and Johnson K. G.: Stress and Animal Welfare, 1st Edition. Published by Chapman & Hall - London, 1993.

Brown, B.W. A review of Nutritional Influences on Reproduction in Boars, Bulls and Rams. Reproduction Nutrition Developpement, 1994; 34: 89-114.

Brown-Borg H. M., Klemcke, H. C. and Blecha, F.: Lymphocyte Proliferative Responses in Neonatal Pigs With High or Low Plasma Cortisol Concentration After Stress Induced by Restraint. American Journal of Veterinary Research, 1993; 54: 2015-2020.

Bruggeman V., Vanmontfort D., Renaville R., Portetelle D. and Decuypere E.: The Effect of Food Intake From Two Weeks of Age to Sexual Maturity on Plasma Growth Hormone, Insulin-like Growth Factor-I, Insulin-like

Growth Factor Binding Proteins, and Thyroid Hormones in Female Broiler Breeder Chickens. General and Comparative Endocrinology, 1997; 107: 212-220.

Burén J. and Eriksson J. W.: Is Insulin Resistance Caused by Defects in Insulin's Target Cells or By a Stressed Mind. Diabetes Metabolism Research and Reviews, 2005; 21: 487-494.

Carenzi C., Bretoni G., Bordi A., Casamassima D., Nanni Costa L., Sevi A. and Verga M.: Punti Critici Relativi al "Benessere Animale" nell'Allevamento Intensivo.. Zootecnia e Nutrizione Animale, 2001; 27: 75-84.

Carroll J. A., Veum T. L. and Matteri R. L.: Endocrine Responses to Weaning and Changes in Post-weaning Diet in the Young Pig. Domestic Animal Endocrinology, 1998; 15: 183-194.

Casanueva F. F., Burguera B., Muruais C. and Dieguez C. Acute Administration of Corticoids: a New and Peculiar Stimulus of Growth Hormone Secretion in Man. Journal of Clinical Endocrinology and Metabolism, 1990; 70: 234-237.

Chan O., Inouye K., Akirav E., Park E., Riddell M. C., Vranic M. and Matthews S. G.: Insulin Alone Increases Hypothalamo-Pituitary-Adrenal Activity, and Diabetes Lowers Peak Stress Responses. Endocrinology, 2005; 146(3): 1382-1390.

Chen H. H., Lin J. H. and Fung H. P.: Serum Acute Phase Proteins and Swine Health Status. Canadian Journal of Veterinary Research, 2003; 67(4): 283-290.

Chilliard Y., Bocquier F. and Doreau M.: Digestive and Metabolic Adaptations of Ruminants to Undernutrition, and Consequences on Reproduction. Reproduction Nutrition Developpement, 1998; 38: 131-152.

Conrad C. D. and McEwen B. S.: Acute Stress Increases Neuropeptide Y mRNA Within the Arcuate Nucleus and Hilus of the Dentate Gyrus. Molecular Brain Research, 2000; 79: 102-109.

Danforth E. Jr and Burger A.: The Role of Thyroid Hormones in the Control of Energy Expenditure. Clinics in Endocrinology and Metabolism, 1984; 13: 581-595.

Dawkins M.: From an Animal's Point of View: Motivation, Fitness and Animal Welfare. Behavior and Brain Science, 1990; 13: l-61.

Diano S., Naftolin F., Goglia F. and Horvath T. L.: Fasting-induced Increase in Type II Iodothyronine Deiodinase Activity and Messenger Ribonucleic Acid Levels is not Reversed by Thyroxine in the Rat Hypothalamus. Endocrinology, 1998; 139: 2879-2884.

Dinan T. G.: Serotonin and the Regulation of the Hypothalamic-pituitary-adrenal Axis Function. Life Sciences, 1996; 58: 1683-1694.

Dobson H. and Smith R.F.: Stress and Reproduction in Farm Animals. Journal of Reproduction and Fertility, 1995; 49 (Suppl.): 451-461.

Drago F., D'Agata V., Iacona T., Spadaro F., Grassi M., Valerio C., Raffaele R., Astuto C., Lauria N. and Vitetta M.: Prolactin as a Protective Factor in Stress-induced Biological Changes. Journal of Clinical Laboratory Analysis, 1989; 3: 340-344.

Dyer C. J., Simmons J. M., Matteri R.L. and Keisler D. H.: Leptin Receptor mRNA is Expressed in Ewe Anterior Pituitary and Adipose Tissue and is Differentially Expressed in Hypothalamic Regions of Well-fed and Feed-restricted Ewes. Domestic Animal Endocrinology, 1997; 14(2): 119-128.

Dyer C. J., Touchette K. J., Carroll J.A., Allee G.L. and Matteri R.L.: Cloning of Porcine Prepro-orexin cDNA and Effects of Synthetic Porcine Orexin-B on Feed Intake in Young Pigs. Domestic Animal Endocrinology, 1999; 16: 145-148.

FAWC: Interim Report on the Animal Welfare Implications of Farm Assurance Schemes. Published by The Farm Animal Welfare Council - London, UK; 2001.

Ferin M.: Neuropeptides, the Stress Response, and the Hypothalamo-pituitary-gonadal Axis in the Female Rhesus Monkey. Annals of the New York Academy of Sciences; 1993; 697: 106-116.

Fink G., Rosie R., Sheward W.J., Thomson E. and Wilson H.: Steroid Control of Central Neuronal Interactions and Function. Journal of Steroid Biochemistry and Molecular Biology, 1991: 40; 123-132.

Forbes J. M.: Environmental Factors Affecting Intake. In: Voluntary Food Intake and Diet Selection in Farm Animals. Edited by Forbes J. M. Published by CAB International - Wallingford, UK; 1995, pp. 332-353.

Fraser A. F. and Broom D. M.: Farm Animal Behaviour and Welfare. Edited by Fraser A. F. and Broom D. M. Published by Saunders - New York, USA, 1990.

Gale S. M., Castracane V. D. and Mantzoros C. S.: Energy Homeostasis, Obesity and Eating Disorders: Recent Advances in Endocrinology. Journal of Nutrition, 2004; 134: 295-298.

Giguere V. and Labrie F.: Additive Effects of Epinephrine and Corticotropin-releasing Factor (CRF) on Adrenocorticotropin Release in Rat Anterior Pituitary Cells. Biochemical and Biophysical Research Communications, 1983; 110: 456-462.

Goldstein D. S., McCarty R., Polinsky R. J. and Kopin I. J.: Relationship Between Plasma Norepinephrine and Sympathetic Neural Activity. Hypertension, 1983; 5: 552-559.

Gonyou H. W.: Why the Study of Animal Behavior Is Associated With the Animal Welfare Issue. Journal of Animal Science, 1994; 72: 2171-2177.

Goya R. G., Sosa Y. E., Console G. M. and Dardenne M.: Altered Thyrotropic and Somatotropic Responses to Environmental Challenges in Congenitally Athymic Mice. Brain Behavior and Immunity, 1995; 9: 79-86.

Guillemin R. and Rosenberg B.: Humoral Hypothalamic Control of Anterior Pituitary: a Study with Combined Tissue Cultures. Endocrinology, 1955; 57: 599-607.

Houseknecht K. L. and Portocarrero C. P.: Leptin and Its Receptors: Regulators of Whole-body Energy Homeostasis. Domestic Animal Endocrinology, 1998; 15(6): 457-475.

Houseknecht K. L., Baile C. A., Matteri R. L. and Spurlock M. E.: The Biology of Leptin: A Review. Journal of Animal Sciences, 1998; 76: 1405-1420.

Jurcovicova J., Kvetnansky R., Dobrakovova M., Jezova D., Kiss A. and Makara, G. B.: Prolactin Response to Immobilization Stress and Hemorrhage: the Effect of Hypothalamic Deafferentations and Posterior Pituitary Denervation. Endocrinology, 1990; 126: 2527-2533.

Juszczak M.: Melatonin Affects the Oxytocin and Prolactin Responses to Stress in Male Rats. Journal of Physiology and Pharmacology, 1998; 49: 151-163.

Kas M. J. H., Bruijnzeel A. W., Haanstra J. R., Wiegant V. M. and Adan R. A. H.: Differential Regulation of Agouti-related Protein and Neuropeptide Y in Hypothalamic Neurons Following a Stressful Event. Journal of Molecular Endocrinology, 2005; 35: 159-164.

Kakizawa S., Kaneko T., Hasegawa S. and Hirano T.: Effects of Feeding, Fasting, Background Adaptation, Acute Stress, and Exhaustive Exercise on the Plasma Somatolactin Concentrations in Rainbow Trout. General and Comparative Endocrinology, 1995; 98: 137-146.

Kelley K. W., Osborne C. A., Evermann J. F., Parish S. M. and Hinrichs D. J.: Whole Blood Leukocyte vs. Separated Mononuclear Cell Blastogenesis in Calves: Time-dependent Changes After Shipping. Canadian Journal of Comparative Medicine, 1981; 45: 249-258.

Kelly D. and Coutts A. G. P.: Development of Digestive and Immunological Function in Neonates: Role of Early Nutrition. Livestock Production Science, 2000; 66: 161-167.

Kilgour R.: The Application of Animal Behavior and the Humane Care of Farm Animals. Journal of Animal Science, 1978; 46: 1478-1484.

Kinouchi T., Koizumi K., Kuwata T. and Yajima T.: Milk-borne Insulin With Trypsin Inhibitor in Milk Induces Pancreatic Amylase Development at the Onset of Weaning in Rats. Journal of Pediatric Gastroenterology and Nutrition, 2000; 30(5): 515-521.

Kirschbaum C., Pirke K. M. and Hellhammer D. H.: The "Trier Social Stress Test" - a Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting. Neuropsychobiology, 1993; 28: 76-81.

Klemcke H. G., Neinaber J. A. and Hahn G. L.: Stressor-associated Alterations in Porcine Plasma Prolactin. Proceedings of the Society for Experimental Biology and Medicine, 1987; 186: 333-341.

Kojima M. and Kangawa K.: Ghrelin: Structure and Function. Physiological Reviews, 2005; 85: 495-522.

Krebs C. J.: Ecology. In: The Experimental Analysis of Distribution and Abundance. Edited by Krebs C. J. Published by Harper and Row - New York, USA. 1972. 694 pp.

Lawrence A. B, Dwyer C. M., Jarvis S. and Roberts D.: Welfare Implications of Dairy Calf and Heifer rearing. In: Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer From Calf to Calving. 60th University of Nottingham Easter School in Agricultural Science, Nottingham, UK. 23rd-24th March, 2004. Published by the Nottingham University Press - Nottingham, UK, 2005; pp. 197-212.

Link H., Dayanithi G. and Gratzl M.: Glucocorticoids Rapidly Inhibit Oxytocin-stimulated Adrenocorticotropin Release From Rat Anterior Pituitary Cells, Without Modifying Intracellular Calcium Transients. Endocrinology, 1993; 132: 873-878.

Loan R.W.: Bovine Respiratory Disease: a Symposium. Published by Texas A&M University Press - College Station, Texas, USA, 1984.

Magnusson U., Wattrang E., Tsuma V. and Fossum C.: Effects of Stress Resulting From Short-term Restraint on In Vitro Functional Capacity of Leukocytes Obtained From Pigs. American Journal of Veterinary Research, 1998; 59: 421-425.

Marti O., Gavalda A., Jolin T. and Armario A.: Acute Stress Attenuates but Does Not Abolish Circadian Rhythmicity of Serum Thyrotropin and Growth Hormone in the Rat. European Journal of Endocrinology, 1996; 135: 703-708.

Mason J. W.: Specificity in the Organization of Neuroendocrine Response Profiles. In: Frontiers in Neurology and Neuroscience Research. Edited by Seeman P. and Brown G. Published by the University of Toronto Press - Toronto, Canada, 1974; p. 68.

Mastronardi C. A., Yu W. H. and McCann S. M.: Comparisons of the Effects of Anesthesia and Stress on Release of Tumor Necrosis Factor–a, Leptin, and Nitric Oxide in Adult Male Rats. Experimental Biology and Medicine, 2001; 226(4): 296-300.

Matteri R. L., Watson J. G. and Moberg G. P.: Stress or Acute Adrenocorticotrophin Treatment Suppresses LHRH-induced LH Release in the Ram. Journal of Reproduction and Fertility, 1984; 72: 385-393.

Matteri R. L., Carroll J. A. and C.J.. Dyer C. J.: Neuroendocrine Responses to Stress. In: The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Edited by Moberg G. P. and Mench J. A. Published by CAB International; 2000: pp. 43-76.

Matthews S. G. and Parrott R. F.: Centrally Administered Vasopressin Modifies Stress Hormone (Cortisol, Prolactin) Secretion in Sheep Under Basal Conditions, During Restraint and Following Intravenous Corticotrophin-releasing Hormone. European Journal of Endocrinology, 1994; 130: 297-301.

Medical Research Council: Animals in Medicine and Science. Published by the Medical Research Council - London, UK; 1999.

McCarthy T. L., Centrella M. and Canalis E.: Cortisol Inhibits the Synthesis of Insulin-like Growth Factor-l in Skeletal Cells. Endocrinology, 1990; 126: 1569-1575.

McCracken B. A., Gaskins H. R., Ruwe-Kaiser P. J., Clasing K. C. and Jewell D. E.: Diet-dependent and Diet-independent Metabolic Responses Underlie Growth Stasis of Pigs at Weaning. Journal of Nutrition, 1995; 11: 2838-2845.

McCusker R. H.: Controlling Insulin-like Growth Factor Activity and the Modulation of Insulin-like Growth Factor Binding Protein and Receptor Binding. Journal of Dairy Science, 1998; 81: 1790-1800.

McEwen B. S.: Influences of Adrenocortical Hormones on Pituitary and Brain Functions. Monographs in Endocrinology, 1979; 12: 467-492.

McEwen B. S. and Sapolsky R. M.: Stress and Cognitive Function. Current Opinions in Neurobiology, 1995; 5: 205-216.

McGlone J. J., Salak J. L., Lumpkin E. A., Nicholson R. I., Gibson M. and Norman R. L.: Shipping Stress and Social Status Effects on Pig Performance, Plasma Cortisol, Natural Killer Cell Activity, and Leukocyte Numbers. Journal of Animal Science, 1993; 71: 888-896.

Mercer J. G., Hoggard N., Williams L. M., Lawrence C. B., Hannah L. T., Morgan P. J. and Trayhurn P.: Coexpression of Leptin Expression and Preproneuropeptide Y mRNA in Arcuate Nucleus of Mouse Hypothalamus. Journal of Neuroendocrinology, 1996; 8: 733-735.

Mercer J. G., Moar K. M., Raynor D. V., Trayhurn P. and Hoggard N.: Regulation of Leptin Receptor and NPY Gene Expression in Hypothalamus of Leptin-treated Obese (ob/ob) and Cold-exposed Lean Mice. FEBS Letters, 1997; 402: 185-188.

Minton J. E., Coppinger T. R., Reddy P. G., Davis W. C. and Blecha, F.: Repeated Restraint and Isolation Stress Alters Adrenal and Lymphocyte Functions and Some Leukocyte Differentiation Antigens in Lambs. Journal of Animal Science, 1992; 70: 1126-1132.

Moberg G. P.: How Behavioral Stress Disrupts the Endocrine Control of Reproduction in Domestic Animals. Journal of Dairy Science, 1991; 74: 304-311.

Murata H., Takahashi H. and Matsumoto H.: The Effects of Road Transportation on Peripheral Blood Lymphocyte Subpopulations, Lymphocyte Blastogenesis and Neutrophil Function in Calves. British Veterinary Journal, 1987; 143: 166-174.

Natelson B. H., Trapp W. N., Adamus J. E., Mittler J. C. and Levine B. E.: Humoral Indices of Stress in Rats. Physiology and Behaviour, 1981; 26: 1049-1054.

Newcomer J. W., Selke G., Melson A. K., Gross J., Vogler G. P. and Dagogojack S.: Dose-dependent Cortisol-induced Increases in Plasma Leptin Concentrations in Healthy Humans. Archives of General Psychiatry, 1998; 55(11): 995-1000.

Nicoll C.S.: Ontogeny and Evolution of Prolactin's Functions. Federation Proceedings, 1980; 39: 2563-2566.

Noguchi T.: Protein Nutrition and Insulin-like Growth Factor System. British Journal of Nutrition, 2000; 84(Suppl. 2): S241-S244.

Ortega-Cerrilla M. E. and Mendoza-Martinez G.: Starch Digestion and Glucose Metabolism in the Ruminant: a Review. Interciencia, 2003; 28: 380-386.

Owens F. N., Dubeski P. and Hanson C. F.: Factors that Alter the Growth and Development of Ruminants. Journal of Animal Science, 1993; 71: 3138-3150.

Peisen J. N., McDonnell J. C., Musroney S. E. and Lumpkin M. D.: Endotoxin-induced Suppression of the Somatotropic Axis is Mediated by Interleukin-1 Beta and Corticotropin-releasing Factor in the Juvenile Rat. Endocrinology, 1995; 136: 3378-3390.

Pessin J. E. and Saltiel A. R.: Signaling Pathways in Insulin Action: Molecular Targets of Insulin Resistance. Journal of Clinical Investigation, 2000; 106(2): 165-169.

Peterson P. K., Chao C. C., Molitor T., Murtaugh M., Strgar F. and Sharp B. M.: Stress and the Pathogenesis of Infectious Disease. Reviews in Infectious Diseases, 1991; 13: 710-720.

Plotsky P. M., Cunningham E. T. and Widmair E. P.: Catecholamine Modulation of Corticotrophin-releasing Factor and Adrenocorticotrophin Secretion. Endocrine Reviews, 1989; 10: 437-458.

Plotsky P. M., Thrivikraman K. V. and Meaney M. J.: Central and Feedback Regulation of Hypothalamic Corticotropin-releasing Factor Secretion. Ciba Foundation Symposium, 1993; 172: 59-75.

Plourde V.: Stress-induced Changes in the Gastrointestinal motor System. Canadian Journal of Gastroenterology, 1999;13: 26A-31A.

Pol F., Courboulay V., Cotte J. P., Martrenchar A., Hay M. and Mormède P.: Urinary Cortisol as an Additional Tool to Assess the Welfare of Pregnant Sows Kept in Two Types of Housing. Veterinary Research, 2002; 33: 13-22.

Porter J. and Jones J.: Effect of Plasma from Hypophyseal-portal Vessel Blood on Adrenal Ascorbic Acid. Endocrinology, 1956; 58: 62-67.

Prolo P., Wong M. and Licinio G.: Leptin. The International Journal of Biochemistry & Cell Biology, 1998; 30: 1285-1290.

Richter S. D., Schurmeyer T. H., Schedlowski M., Hadicke A., Tewes U., Schmidt R. E. and Wagner T. O.: Time Kinetics of the Endocrine Response to Acute Psychological Stress. Journal of Clinical Endocrinology and Metabolism, 1996; 81: 1956-1960.

Rivest S. and Rivier C.: The Role of Corticotropin-releasing Factor and Interleukin-1 in the Regulation of Neurons Controlling Reproductive Functions. Endocrine Reviews, 1995; 16: 177-199.

Rivier C. and Rivest S.: Effect of Stress on the Activity of the Hypothalamic-pituitary-gonadal Axis: Peripheral and Central Mechanisms. Biology of Reproduction, 1991; 45: 523-532.

Roth J. A. and Kaeberle M. L.: Effect of Glucocorticoids on the Bovine Immune System. Journal of the American Veterinary Medical Association, 1982; 180: 894-901.

Sakamoto K., Wakabayashi I., Yoshimoto S., Masui H. and Katsuno S.: Effects of Physical Exercise and Cold Stimulation on Serum Testosterone Level in Man. Japanese Journal of Hygiene, 1991; 46: 635-638.

Saffran M., Schally A. and Benfey B.: Stimulation of the Release of Corticotropin from the Adrenohypophysis by a Neurohypophyseal Factor. Endocrinology, 1955; 57: 439-444.

Sakurai T., Amemiya A., Ishii M., Matsuzaki I., Chemilli R. M., Tanaka H., Williams S. C., Richardson J. A., Kozlowski G. P., Wilson S., Arch J. R. S., Buckingham R. E., Haynes A. C., Carr S. A., Annan R. S., McNulty D. E., Liu W. S., Terrett J. A., Elshourbagy N. A., Bergsrna D. J. and Yanagisawa M.: Orexins and Orexin Receptors: a Family of Hypothalamic Neuropeptides and G protein-coupled Receptors that Regulate Feeding Behavior. Cell, 1998; 92: 573-585.

Samuels M. H. and McDaniel P. A.: Thyrotropin Levels During Hydrocortisone Infusions That Mimic Fasting-induced Cortisol Elevations:

a Clinical Research Center Study. Journal of Clinical Endocrinology and Metabolism, 1997; 82: 3700-3704.

Schafroth U., Godang K., Ueland T. and Bollerslev J.: Leptin Response to Endogenous Acute Stress is Independent of Pituitary Function. European Journal of Endocrinology, 2001; 145: 295-301.

Schillo K. K.: Effects of Dietary Energy on Control of Luteinizing Hormone Secretion in Cattle and Sheep. Journal of Animal Science, 1992; 70: 1271-1282.

Schoonmaker J. P., Loerch S. C., Fluharty F. L., Turner T. B., Moeller S. J., Rossi J. E., Dayton W. R., Hathaway M. R., Wulf D. M.: Effect of an Accelerated Finishing Program on Performance, Carcass Characteristics and Circulating Insulin-like Growth Factor I Concentration of Early-weaned Bulls and Steers. Journal of Animal Science, 2002; 80: 900-910.

Seggie J. and Brown G. M.: Profiles of Hormone Stress Response: Recruitment or Pathway Specificity. In: Brain Neurotransmitters and Hormones. Edited by Collu R., Ducharme J. R., Barbeau A. and Tolis G. Published by Raven Press - New York, USA; 1982: p. 277.

Selye H. H. B.: A Syndrome Produced by Diverse Nocuous Agents. Nature, 1936; 138(7): 32.

Selye H. H. B.: The Effect of Adaptation to Various Damaging Agents in the Female Sex Organs in the Rat. Endocrinology, 1939; 25: 615-624.

Selye H. H. B.: The General-adaptation-syndrome and the Diseases of Adaptation. Southern Medicine & Surgery, 1951; 113(10): 315-323.

Selye H. H. B.: Stress and Distress. Comprehensive Therapy, 1995; 1: 9-13.

Siegel R. A., Weidenfeld J., Feldman S., Conforti N. and Chowers I.: Neural Pathways Mediating Basal and Stress-induced Secretion of Luteinizing Hormone, Follicle-stimulating Hormone, and Testosterone in the Rat. Endocrinology, 1981; 108: 2302-2307.

Sheridan J. F., Dobbs C., Jung J., Chu X., Konstantinos A., Padgett D. and Glaser R.: Stress-induced Neuroendocrine Modulation of Viral

Pathogenesis and Immunity. Annals of the New York Academy of Sciences, 1998; 840: 803-808.

Stratakis C. A. and Chrousos P.: Neuroendocrinology and Pathophysiology of the Stress System. Annals of the New York Academy of Sciences, 1995; 771: 1-18.

Stratakis C. A., Gold P. W. and Chrousos G. P.: Neuroendocrinology of Stress: Implications for Growth and Development. Hormone Research, 1995; 43: 162-167.

Straus D. S.: Nutritional Regulation of Hormones and Growth Factors that Control Mammalian Growth. Federation of American Societies for Experimental Biology Journal, 1994; 8: 6-12.

Swanson J. C.: Farm Animal Well-Being and Intensive Production Systems. Journal of Animal Science, 1995; 73: 2744-2751.

Tatemoto K., Carlquist M. and Mutt V.: Neuropeptide Y - a Novel Brain Peptide with Structural Similarities to Peptide YY and Pancreatic Polypeptide. Nature, 1982; 296: 659-660.

Trayhurn P., Duncan J. S. and Rayner D. V.: Acute Cold-induced Suppression of ob (Obese) Gene Expression in White Adipose Tissue of Mice: Mediation by the Sympathetic System. Biochemical Journal, 1995; 311(3): 729-733.

Ueta Y., Ozaki Y., Saito J. and Onaka T.: Involvement of Novel Feeding-related Peptides in Neuroendocrine Response to Stress. Experimental Biology and Medicine, 2003; 228: 1168-1174.

Vance M. L., Hartman M. L. and Thorner M. O.: Growth Hormone and Nutrition. Hormone Research, 1992; 38: 85-88.

Van de Kar L. D., Richardson-Morton K. D. and Rittenhouse P. A.: Stress: Neuroendocrine and Pharmacological Mechanisms. In: Methods and Achievements in Experimental Pathology, vol. 14. Edited by Jasmin G. and Cantin M. Published by Karger - Basel, Switzerland; 1991: pp. 133-173.

Van den Berghe G. and de Zegher F.: Anterior Pituitary Function During Critical Illness and Dopamine Treatment. Critical Care Medicine, 1996; 24: 1580-1590.

Verga M.: Intensive Rabbit Breeding and Welfare: Development of Research, Trends and Apllications. World Rabbit Science, 2000; 8(Suppl. 1, Vol. B): 491-509. (Proceedings of 7th W. R. S. A. Congress, Valencia, Spain. Invited Paper).

Verga M. and Ferrante V.: La Ricerca su Benessere e Adattamento nel Coniglio. Rivista di Coniglicoltura, 2002; 2: 31-39.

Vitali A., Magistrelli D., Rosi F., Azevedo J., Bernabucci U. and Ronchi B.: Leptin and the Onset of Puberty in Goat. Italian Journal of Animal Science, 2005, 4(Suppl. 2): 383-385. (Proceeding of the ASPA XVI Congress, Torino, June 28-30, 2005).

Warming E.: Plantesamfund - Grundtræk af den Økologiske Plantegeografi. Published by Philipsens Forlag P.G. – Kjøbenhavn, Denmark, 1895; 335 pp.

Weigent D. A.: lmmunoregulatory Properties of Growth Hormone and Prolactin. Pharmacological Therapeutics, 1996; 69: 237-257.

Weissman C.: The Metabolic Response to Stress: an Overview and Update. Anesthesiology, 1990; 73: 308-327.

Wenk C.: Environmental Effects on Nutrient and Energy Metabolism in Pigs. Archiv fur Tierernahrung, 1998; 51: 211-224.

Wilczynski A., Wang X. S., Bauzo R. M., Xiang Z., Shaw A. M., Millard W. J., Richards N. G., Edison A. S. and Haskell-Luevano C.: Structural Characterization and Pharmacology of a Potent (Cys101-Cys119, Cys110-Cys117) Bicyclic Agouti-related Protein (AgRP) Melanocortin Receptor Antagonist. Journal of Medicinal Chemistry, November 4th 2004; 47(23): 5662-5673.

Williams C. L., Villar R. G., Peterson J. M. and Burks T. F.: Stress-induced Changes in Intestinal Transit in the Rat: a Model for Irritable Bowel Syndrome. Gastroenterology, 1988; 94: 611-621.

Xiao E., Xia-Zhang L., Thornell D., Shalts E. and Ferin M.: The Luteinizing Hormone but not the Cortisol Response to Arginine Vasopressin is Prevented by Naloxone and a Corticotropin-releasing Hormone Antagonist in the Ovariectomized Rhesus Monkey. Neuroendocrinology, 1996; 64: 225-232.

Zhang Y., Proenca R., Maffei M., Barone M., Leopold L. and Friedman J. M.: Positional Cloning of the Mouse Obese Gene and Its Human Homologue. Nature, 1994; 372: 425-432.

2 EXPERIMENTAL DESIGN

2.1 Aim of the Study

As previously stated, weaning is a crucial period in the life of farm animals (Owens et al., 1993; McCracken et al., 1995; Schoonmaker et al., 2002; Lawrence et al., 2005).

The process of transitioning animals from their neonatal reliance on nutrients supplied from milk to nutrients supplied from hay and concentrate or grain-based diets is of substantial economic importance to the producer (Baldwin et al., 2004).

The choice of the weaning protocol is particularly important for ruminants, since this period is characterized not just by the adaptation of digestive activity, but also by the development of rumen function. The events surrounding the development of the rumen are considered the most dramatic physiological challenges to young ruminants (Baldwin et al., 2004).

The rumen epithelium is responsible for several physiologically important functions, including absorption, transport, short-chain fatty acid metabolism, and protection (Galfi et al., 1991). Its maturation is the result of differential expression of numerous genes regulating both physical and metabolic characteristics of the tissue (Baldwin et al., 2004). While the most dramatic physical changes occurring during development are associated with the rumen epithelium, changes in intestinal mass and metabolism are also realized in response to dietary changes (Baldwin et al., 2004).

According to the importance of weaning in the life of farm animals the aim of the present study was to evaluate the welfare status of goat kids during the transition from pre-ruminant to ruminant state, trough the analysis of the endocrine/metabolic asset. Another aim was to determine the activity of internal organs (such as liver and pancreas), in order to estimate the functional development of young animals at the time of weaning.

The knowledge of the endocrine and metabolic asset at weaning could also help to remove those stressors that can alter the development, welfare, productivity and even the environmental impact of farm animals.

2.2 Materials and Methods

2.2.1 Animals

Figure 1. A Saanen goat kid.

Eleven Saanen goat kids were separated from their mothers, immediately after birth, and randomly assigned to one of two groups: MILK (6 animals) and WMIX (5 animals). Mean body weight (BW) was 3.72 ± 0.57 kg (mean ± SD) in the MILK group and 3.60 ± 0.47 kg in the WMIX group. The kids of the both groups were housed in separated steel boxes (2.0 m x 1.3 m) provided with straw bedding. The boxes were maintained under identical lighting (13.8 ± 0.9 h/d) and temperature (16.2 ± 6.5 °C) conditions over the study period. At age 50 days all animals were slaughtered five hours after the first meal of the day. The animals used in the present study were cared for and slaughtered in accordance with the guidelines established by the European Union and the Italian Ministry of Health.

2.2.2 Diets

All animals were fed colostrum for the first three days of life. The MILK group then received *ad libitum* goat milk for the rest of the study period (to age 50 days). The WMIX group received *ad libitum* goat milk to age 29 days and then underwent weaning in which milk was progressively replaced by solid feed. Specifically, from age 30 to 36 days the WMIX group received 2.5 l/d per head of milk plus *ad libitum* weaning mixture. From age 37 to 47 days the milk was gradually reduced to 1.0 l/d per head. On the 48th day, milk was completely withdrawn. All animals were fed twice a day (9:00 am and 7:00 pm).

The weaning feed mixture consisted of grass hay (30 %), dehydrated alfalfa hay (10 %), steam-flaked corn (19 %), corn gluten meal (3 %), dried sugar beet pulp (8 %), soybean meal (15 %), sunflower seeds (4 %), sugar cane molasses (4 %) and mineral/vitamin supplement (7 %).

2.2.3 Measures and Samples

During the experimental period, the total consumption per group was recorded daily. Individual BWs were recorded weekly, before the first meal of the day.

Every day, all the kids were observed during expectation, administration and consumption of feeds, in order to observe eventual abnormal behaviours.

On days 23, 30, 37 and 44 of life, samples of the fed milk were collected from the morning and from the evening feedings. On days 30, 37 and 44, also weaning mixture samples were collected.

At 23, 30, 37, 44 and 50 days of age, jugular vein blood samples were taken from each animal before the first meal of the day. The blood was collected into vacuum tubes containing K_3EDTA and aprotin (Trasylol, Bayer, 50 µl/ml) and immediately centrifuged at 2000 x g for 15 minutes. The plasma obtained was stored at –20 °C pending analysis.

At slaughtering, liver and carcass weights were recorded and slaughtering yield was measured. With the term "carcass" it is considered the eviscerated animal after removal of blood and skin: it includes the head. The yield is the carcass weight over the live weight, expressed as percentage.

At slaughtering, samples of liver and pancreas were taken, cooled in liquid nitrogen and stored at –80 °C until analysis.

2.2.4 Analysis

Immediately after sampling, milk was analyzed for dry matter, crude protein, fat and lactose, by IDF-approved methods (Hopkin, 1984). Weaning

mixture samples were analyzed for dry matter, crude protein, ether extract, and starch, according to AOAC guidelines (AOAC, 1990).

Plasma was analyzed for glucose (Wako Chemicals - Neuss, Germany), free amino acids (Goodwin, 1968), total protein, urea, creatinine (Giesse Diagnostics - Colle Prenestino, Rome, Italy) and albumin (Boehringer Mannheim - Mannheim, Germany) by spectrophotometric methods. On plasma, leptin (Multispecies Leptin RIA Kit, Linco Research - St. Charles, MO, USA), insulin (Insik-5, Dia Sorin - Saluggia, Italy) and IGF-1 (Total IGF-1 With Extraction, Diagnostics System Laboratories - Webster, TX, USA) were determined by radio-immune assays (RIA); ghrelin was determined by enzyme-linked immunosorbent assay (ELISA) (Total Ghrelin, Diagnostics System Laboratories - Webster, TX, USA). Plasma activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Boehringer Mannheim - Mannheim, Germany) and α-amylase (Giesse Diagnostics - Colle Prenestino, Rome, Italy) were analyzed by enzymatic-spectrophotometric methods. Finally, plasma was tested for cortisol (DSL-2000, Diagnostic System Laboratories - Webster, TX, USA) and haptoglobin (Giesse Diagnostics - Colle Prenestino, Rome, Italy) content by RIA and turbidimetric method, respectively.

Liver samples were analyzed for DNA, RNA, phospholipids and soluble protein content, ALT and AST activity. According to Munro and Fleck (1966), 200 mg of liver were homogenated in 4 ml of ice-cold distilled water, added with 2.5 ml of 0.6 N $HClO_4$ and incubated 10 minutes at room temperature, after mixing. The precipitate formed after incubation was separated by centrifugation for 5 minutes at 1500 x g and then washed twice with 2 ml of 0.2 N $HClO_4$. After that, the precipitate was added with 4 ml of 0.3 N KOH and incubated for 1 hour at 37 °C. The obtained solution was added with 2.5 ml of 1.2 N $HClO_4$ and incubated on ice for 10 minutes to allow complete precipitation. The precipitate was separated by centrifugation and washed twice with 5 ml of 0.2 N $HClO_4$.

For the analysis of RNA in liver, washings and the supernatant were combined and further 10 ml of 0.6 N $HClO_4$ were added and the volume was made up with distilled water to 100 ml. The extinction (E) of the solution was measured at 232 nm and 260 nm. The content of RNA phosphous was calculated with the formula: P_{RNA} (μg/ml) = 3.40 E_{260} – 1.44 E_{232} and RNA content was calculated considering P_{RNA} as the 6.4 % of the tissual RNA (Munro and Fleck, 1966).

For the analysis of DNA content in liver, the precipitate, obtained after incubation on ice for 10 minutes, was dissolved in 5 ml of 0.3 N KOH and added with 12 ml of 0.3 N KOH. Then the volume was made up to 50 ml with distilled water. 2 ml of the solution were added with a 0.04 % indole solution and 1 ml of concentrated HCl and mixed. The mixture was placed in a boiling

water bath for 10 minutes, then cooled in running water. The mixture was extracted 3 times with $CHCl_3$ and the aqueous layer was read at 490 nm. The amount of DNA was obtained from a standard curve of different concentrations of thymus DNA (Munro and Fleck, 1966).

For the analysis of suluble protein and phospholipids content and ALT and AST activity in liver, 250 mg of fresh tissue were homogenized in 1 ml of water with an Ultra Turrax homogenizer. 0.5 ml of the suspension were transferred in a centrifuge tube and then centrifuged for 5 minutes at 800 x g. The supernatant was analyzed for soluble protein content (Pierce Biotecnology Inc. – Rockford, IL, USA) and ALT and AST activity (Boehringer Mannheim - Mannheim, Germany) with spectrophotometric methods. ALT catalyzes the conversion of alanine to pyruvate. Pyruvate, in presence of NADH is converted into lactate by the enzyme lactate dehydrogenase. The estintion of NADH can be measured spectrophotometrically at 340 nm and its variation per minute is properly related to the concentration of ALT. Instead, AST is an enzyme that catalyzes the conversion of aspartic acid to oxaloacetate, which is rapidely converted into malate, in presence of NADH, by the enzyme malate dehydrogenase. Once again, the measure of the variation of the extinction per minute of NADH gives the concentration of AST. Transaminase activity is usually expressed in international unit (IU/l), which is considered as the amount of enzyme that converts 1 µmol of amino acids into oxo acids per minute at 37 °C.

According to Folch (1957), 0.5 ml of the homogenized suspension were extracted twice with 10 ml of 2:1 mixture of $CHCl_3$-CH_3OH. The two extracts were added with 4.5 ml of 0.73 % NaCl solution and then centrifuged for 10 minutes at 1000 x g. After removing and discarding the upper layer, the volume was made up to 25 ml with methanol. Phosphorus was determined with Allen's procedure (1940). 3 ml of the extract were evaporated to dryness at 100 °C and digested with 0.4 ml of 72 % $HClO_4$ at 200 °C for 1 hour. After cooling, the digested extract was added with 4 ml of distilled water, 0.4 ml of 1 % amidol solution in 20 % sodium metabisulfite and 0.2 ml of 8.3 % ammonium molybdate solution, mixing after each addition. After 5 minutes, the obtained mixture was read at 725 nm. The amount of phosphorous was obtained from a standard curve and phospholipids content was calculated considering phosphorus as the 4 % of phospholipids.

From pancreas, zymogen granules were extracted by Kinouchi's method (Kinouchi et al., 1998). According to this method, 100 mg of pancreas were homogenized with Ultra Turrax homogenizer in 2 ml of ice-cold buffer (0.25 M sucrose, 5 mM MOPS, and 0.1 mM $MgSO_4$, pH 7.0). The homogenate was centrifuged at 150 x g for 10 minutes to remove unbroken cells and nuclei. The supernatant was re-centrifuged at 1300 x g for 10 minutes. The white layer at

the bottom was suspended in 0.15 M Tris HCl (pH 8.2) containing 0.01 M $CaCl_2$ and then briefly sonicated to break the membranes of the zymogen granules. The suspension was centrifuged at 3000 x g for 5 minutes and the resultant supernatant was analyzed for zymogen content (Pierce Biotecnology Inc. – Rockford, IL, USA) and for α-amylase activity (Giesse Diagnostics Snc, Colle Prenestino – Roma, Italy). This method is based on the chromogenic substrate 2-chloro-4-nitrophenyl maltotrioside. The reaction of amylase with this substrate results in the formation of 2-chloro-4-nitrophenol, that can be spectro-photometrically measured at 405 nm. One IU of amylase activity was defined as the amount of enzyme which released 1 μmol of dichlorophenol per minute at 37 °C.

Pancreas samples were also analyzed for DNA and RNA content, as previously described.

2.2.5 Statistical Analysis

Data obtained were evaluated by the two-ways ANOVA procedure (analysis of variance) of SAS (SAS, 1999), considering the effects of weaning and of period as the independent variables. Differences were considered significant when $P<0.05$ and highly significant when $P<0.01$.

The results from analysis of the experimental feeds will be presented as mean value ± standard deviation (SD). All the other results will be presented as mean value ± standard error of means (SEM).

2.3 Literature Cited

Allen R. J. L.: The Estimation of Phosphorus. Biochemical Journal, 1940; 34: 858-865.

AOAC: Official Methods of Analysis (15th Edition). Published by the Association of Official Analysis Chemists - Washington DC, USA, 1990.

Baldwin R. L., McLeod K. R., Klotz J. L. and Heitmann R. N.: Rumen Development, Intestinal Growth and Hepatic Metabolism in The Pre- and Postweaning Ruminant. Journal of Dairy Science, 2004; 87(E. Suppl.): E55–E65.

Colombo C., Biffi A., Agosti M., Crosignani A., Bennato V. and Marini A.: Pancreatic Development in Newborn Guinea Pigs Fed Intact or Low-

hydrolyzed Protein Formulas. Journal of Pediatric Gastroenterology and Nutrition, 2005; 41: 644-649.

Folch J., Lees M. and Stanley G. H. S.: A Simple Method for the Isolation and Purification of Total Lipides From Animal Tissues. Journal of Biological Chemistry, 1957; 226: 497-509.

Galfi P., Neogrady S. and Sakata T.: Effects of Volatile Fatty Acids on the Epithelial Cell Proliferation of the Digestive Tract and its Hormonal Mediation. In: Physiological Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology. Edited by Tsuda T., Sasaki Y. and Kawashima R. Published by Academic Press, Inc. - San Diego, CA., USA; 1991, pp 49-59.

Goodwin J. F.: The Colorimetric Estimation of Plasma Amino Nitrogen With DNFB. Clinical Chemistry, 1968; 14(11): 1080-1090.

Hopkin E., Boelsma S. and Tuinstra-Lauwaars M.: Inventory of IDF/ISO/AOAC Adopted Methods of Analysis for Milk and Milk Products. Published by the International Dairy Federation - Paris, France, 1984.

Kinouchi T., Koizumi K., Kuwata T. and Yajima T.: Crucial Role of Milk-borne Insulin in the Development of Pancreatic Amylase at the Onset of Weaning in Rats. American Journal of Physiology, 1998; 275: 1958-1967.

Lawrence A. B, Dwyer C. M., Jarvis S. and Roberts D.: Welfare Implications of Dairy Calf and Heifer rearing. In: Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer From Calf to Calving. 60th University of Nottingham Easter School in Agricultural Science, Nottingham, UK. 23rd-24th March, 2004. Published by the Nottingham University Press - Nottingham, UK, 2005; pp. 197-212.

McCracken B. A., Gaskins H. R., Ruwe-Kaiser P. J., Clasing K. C. and Jewell D. E.: Diet-dependent and Diet-independent Metabolic Responses Underlie Growth Stasis of Pigs at Weaning. Journal of Nutrition, 1995; 11: 2838-2845.

Munro H. N. and Fleck A.: The Determination of Nucleic Acids. Methods in Biochemistry Analalysis, 1966; 14: 113-176.

Owens F. N., Dubeski P. and Hanson C. F.: Factors that Alter the Growth and Development of Ruminants. Journal of Animal Science, 1993; 71: 3138-3150.

SAS: User's Guide: Statistics Version 7.03. Published by the SAS Institute Inc. - Cary, NC, USA, 1999.

Schoonmaker J. P., Loerch S. C., Fluharty F. L., Turner T. B., Moeller S. J., Rossi J. E., Dayton W. R., Hathaway M. R. and Wulf D. M.: Effect of an Accelerated Finishing Program on Performance, Carcass Characteristics and Circulating Insulin-like Growth Factor I Concentration of Early-weaned Bulls and Steers. Journal of Animal Science, 2002; 80: 900-910.

3 RESULTS

Results will be presented in form of tables and graphs. Significant differences will be indicated with a single asterisk (*), highly significant differences will be indicated with a double asterisk (**). The MILK group will be represented in blue, while the WMIX group will be indicated with the yellow colour. Bars indicate SEM.

Attention will focus chiefly on day 50 of age.

3.1 Diets

Goat's milk composition and weaning mixture composition are expressed in table 1 and 2, respectively. Data are expressed as fed and on dry matter (DM). All values are means ± SD. n = 8 for milk; n = 3 for weaning mixture.

The energy supplied by the goat's milk was calculated from the mean value of nutrients, according with Sandrucci et al. (1995). The energy supplied by the weaning mixture was calculated from the mean value of nutrients, according with Ewan (1989).

As observed, the glucose source for WMIX kids changed from lactose to starch during the transition to the solid diet.

Table 1. Goat's milk composition and gross energy.

	As Fed	On Dry Matter
Dry Matter (%)	12.2 ± 0.64	
Crude Protein (%)	3.22 ± 0.09	26.3 ± 0.72
Fat (%)	3.68 ± 0.48	29.5 ± 3.93
Lactose (%)	4.73 ± 0.09	38.6 ± 0.75
Gross Energy (kJ/kg)	2.72	22.2

Table 2. Weaning mixture composition and gross energy.

	As Fed	On Dry Matter
Dry Matter (%)	88.8 ± 0.09	
Crude Protein (%)	14.6 ± 1.44	15.9 ± 2.38
Fat (%)	3.93 ± 1.17	4.43 ± 1.32
Starch (%)	16.0 ± 0.75	18.9 ± 0.84
Gross Energy (kJ/kg)	16.1	18.2

3.2 Abnormal Behaviours

During the entire experimental period, no case of aggression, neither injurious behaviours, nor stereotypies has been recorded.

3.3 Ingestion and Body Weights

As observed in figure 2, as long as all the kids were fed milk only, dry matter intake (DMI) was similar in both groups. Seven days after the addition of solid feed, DMI in the WMIX group began to decrease compared to the MILK group. From day 37 of age, DMI began to be significantly different ($P<0.01$) between the experimental groups. DMI has been calculated as mean for each kid.

Despite the difference observed in DMI, starting from day 37 of age, body weight did not differ between the two groups throughout the entire study period (figure 3).

Figure 2. DMI expressed as gram per head per day. Red line indicates the change of WMIX diet.

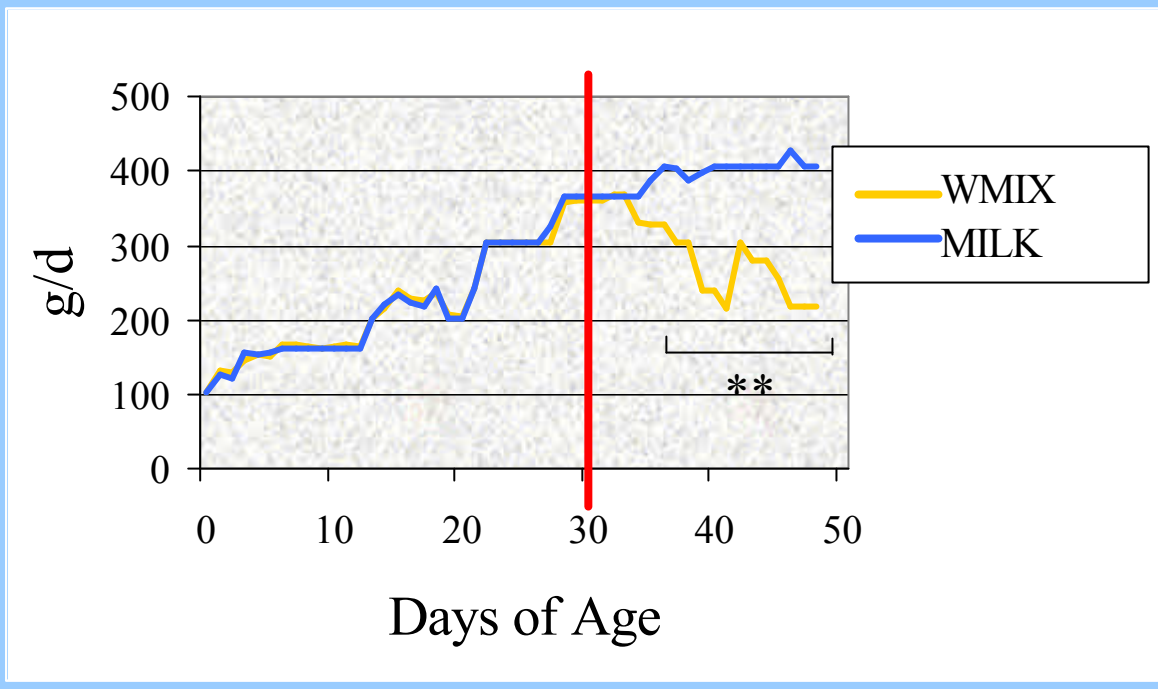
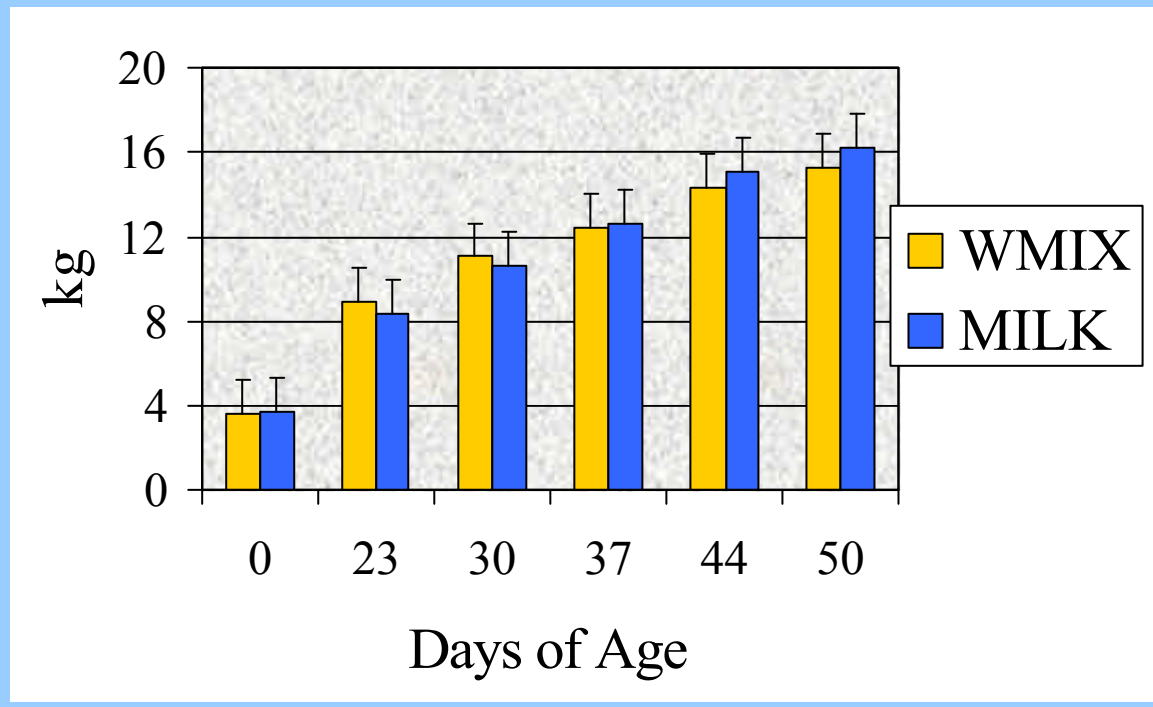


Figure 3. Mean BW.



3.4 Metabolic Traits

Data obtained for glucose, free amino acids, total protein, albumin, urea and creatinine are shown in figure 4, 5, 6, 7, 8 and 9, respectively. During the last four weeks of life, the WMIX group had significantly lower plasma levels of glucose (6.55 mM vs. 6.90; SEM=0.27, $P<0.05$) and free amino acids (4.89 mM vs. 5.27 mM; SEM=0.17, $P<0.01$) and significantly higher plasma creatinine (71.7 µM vs. 69.3 µM; SEM=1.44, $P<0.05$) than the MILK group. In the course of the same four weeks, overall means of plasma total protein, albumin and urea were not different between the groups.

The largest differences were found at age 50 days (two days after the completion of weaning), when glucose (figure 4), free amino acids (figure 5) and urea (figure 8) were significantly lower, and creatinine (figure 9) was significantly higher in the WMIX than the MILK group. Plasma free amino acids and urea began to differ ($P<0.05$) between the experimental groups on day 44 of age (figure 5 and 8, respectively).

Figure 4. Plasma glucose.

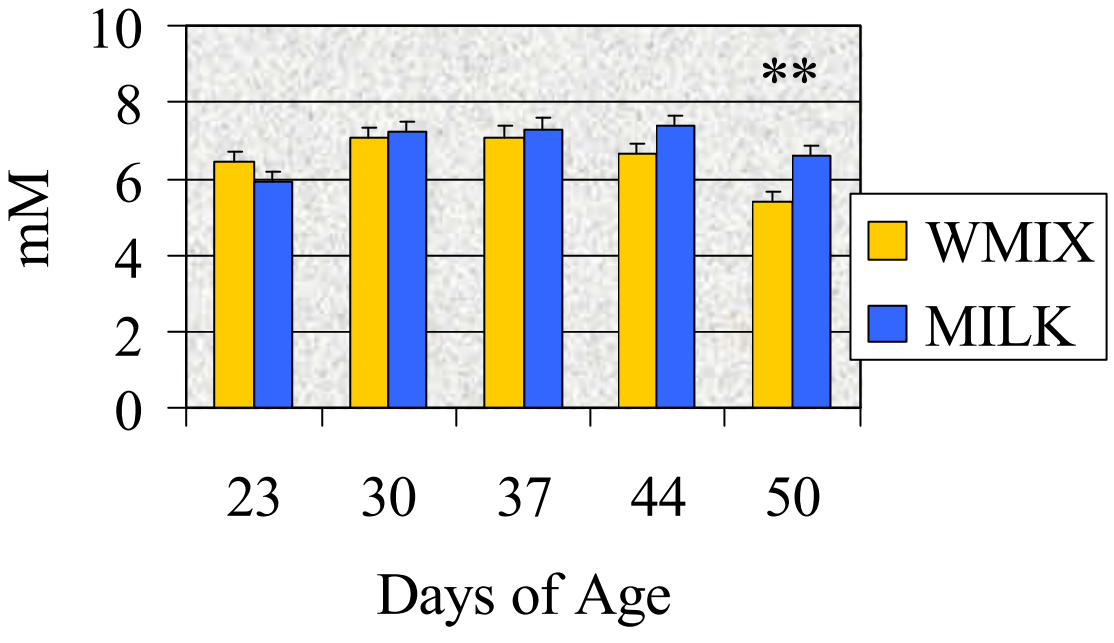


Figure 5. Plasma free amino acids.

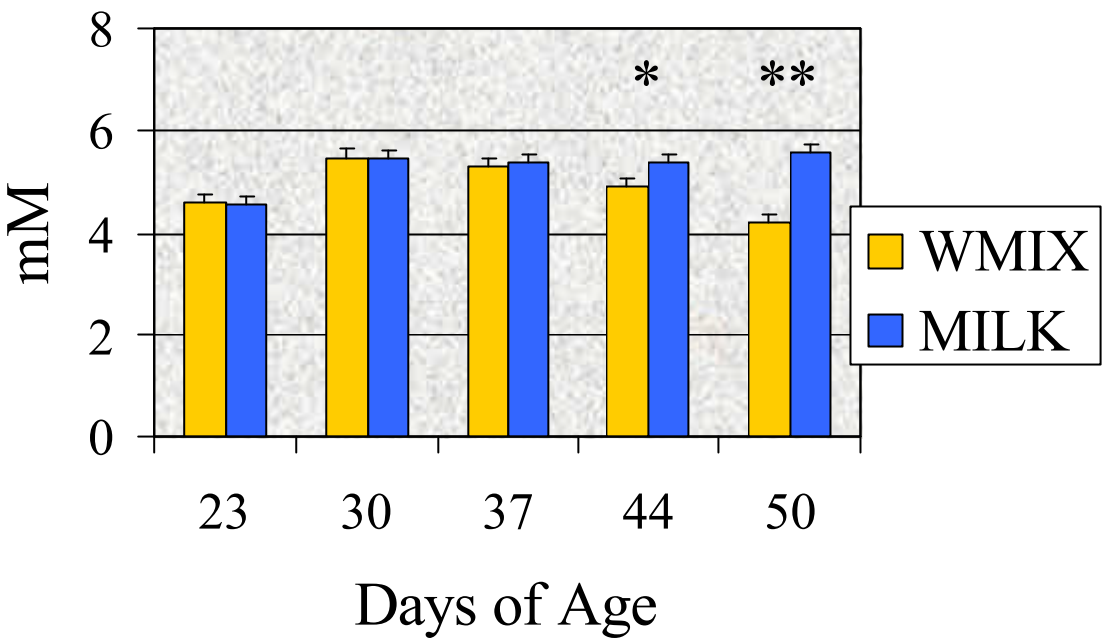


Figure 6. Plasma total protein.

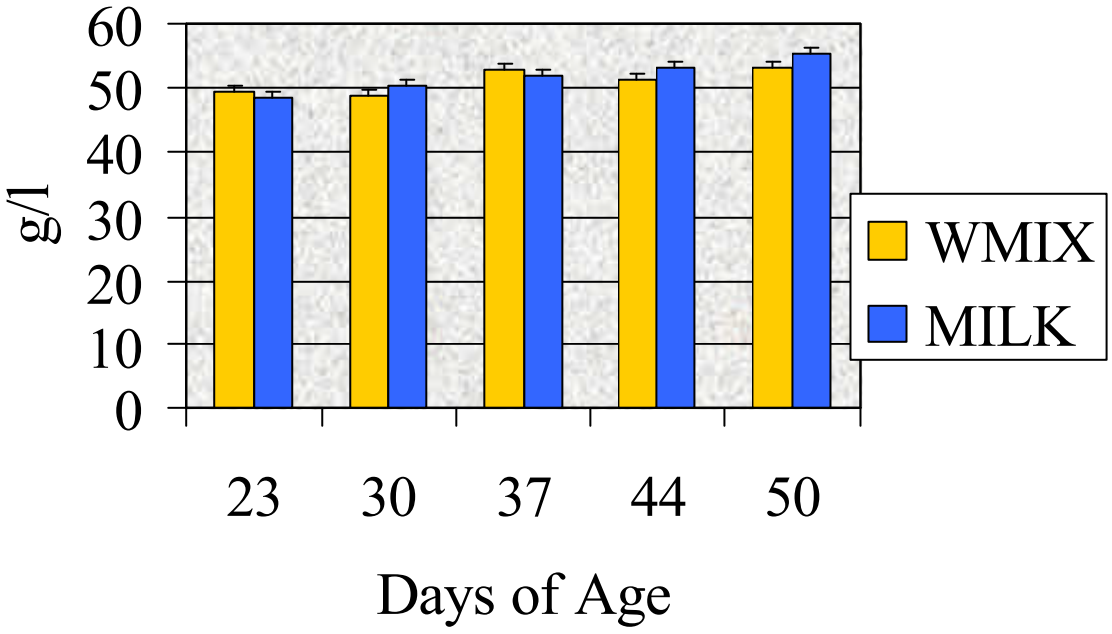


Figure 7. Plasma albumin.

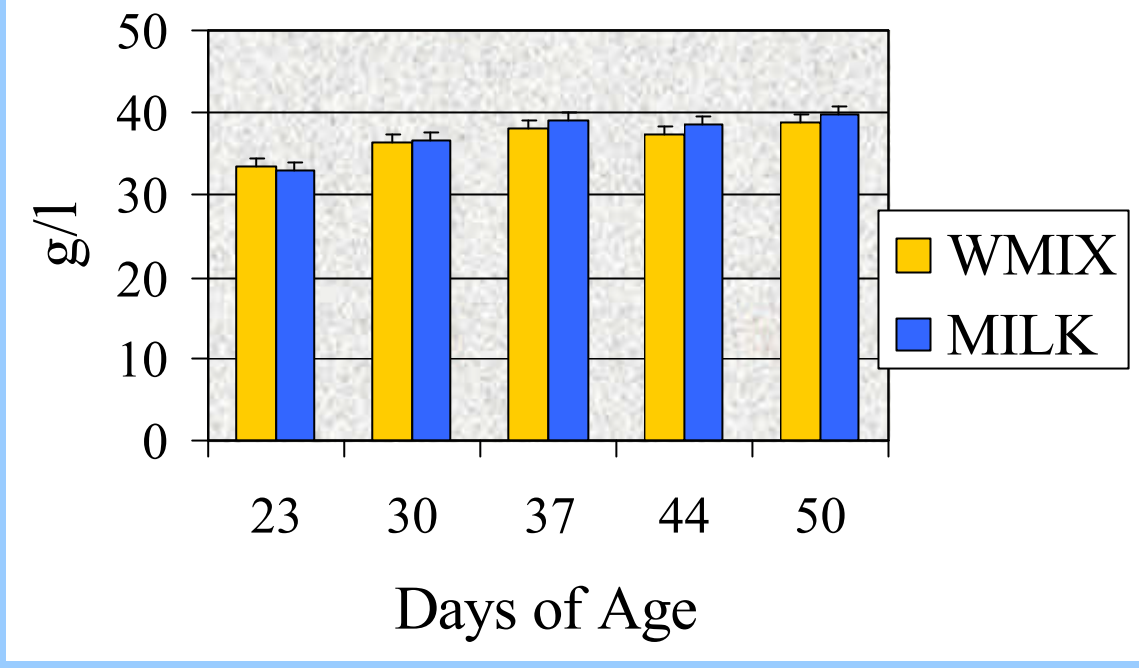


Figure 8. Plasma urea.

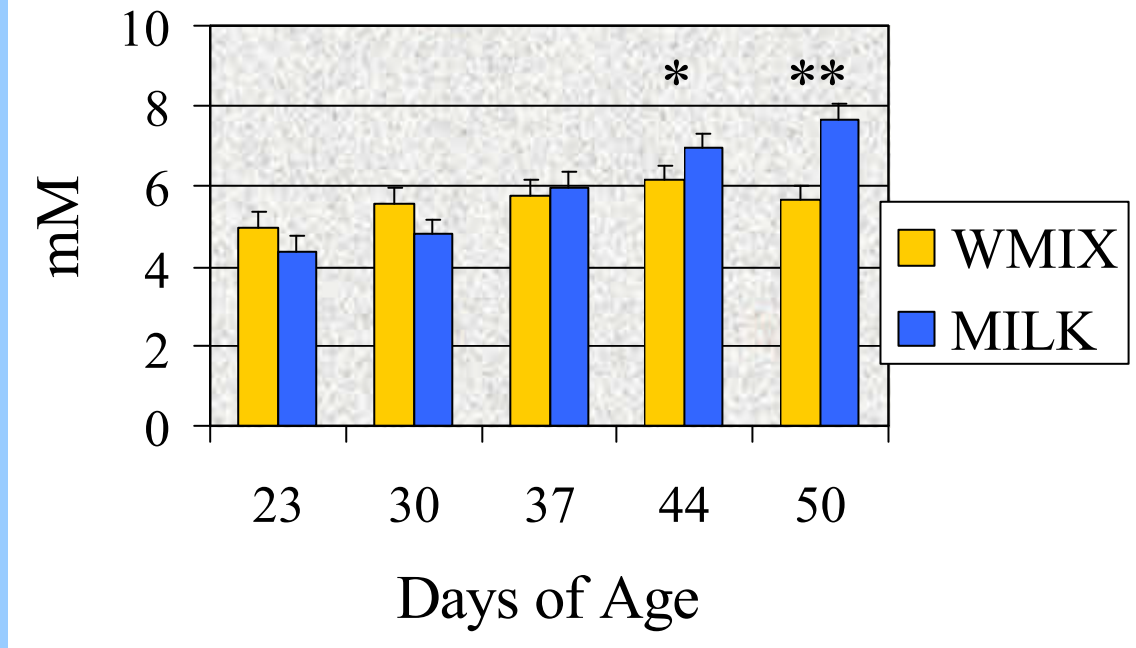
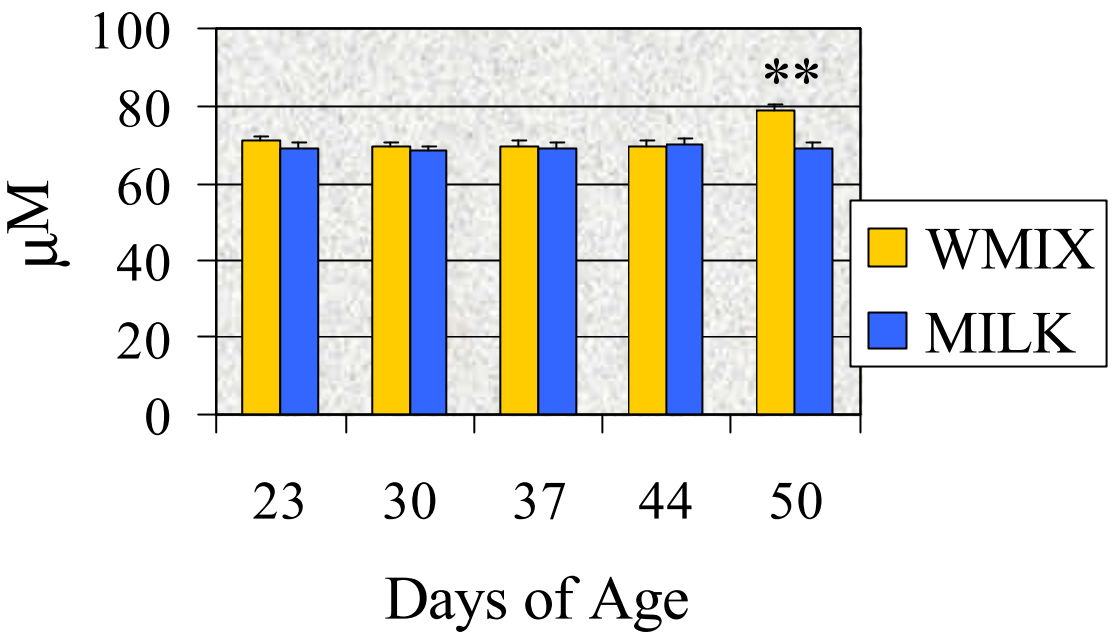


Figure 9. Plasma creatinine.



3.5 Plasma Hormones

Data obtained for leptin, insulin, IGF-1 and ghrelin are shown in figure 10, 11, 12 and 13 respectively. During the last four weeks of life there was no difference between the two experimental groups for the hormones analyzed.

It is worthy of note to observe that plasma leptin level constantly decreased ($P<0.05$) in both groups, between the third and the sixth week of life (figure 10). By contrast, IGF-1 increased ($P<0.01$), until the sixth week, then decreased ($P<0.01$) in the WMIX kids, during the last week of the experimental period (figure 12).

At 50 days of age, plasma insulin and IGF-1 were more than three times lower ($P<0.05$) (figure 10), and ghrelin was significantly higher ($P<0.05$) in the WMIX than the MILK group (figure 12).

Figure 10. Plasma leptin.

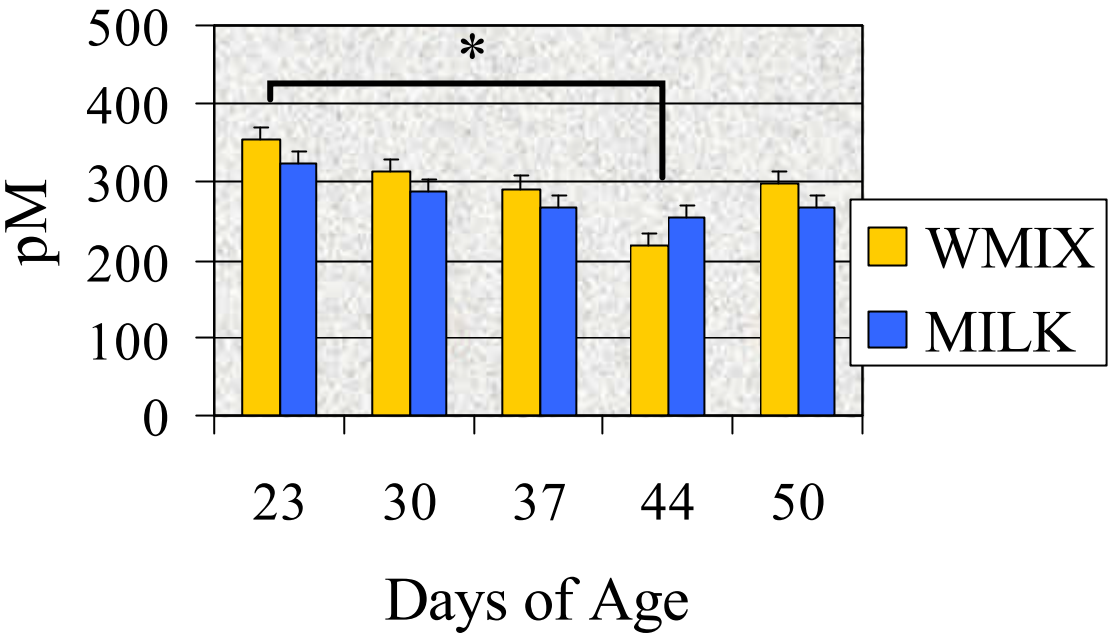


Figure 11. Plasma insulin.

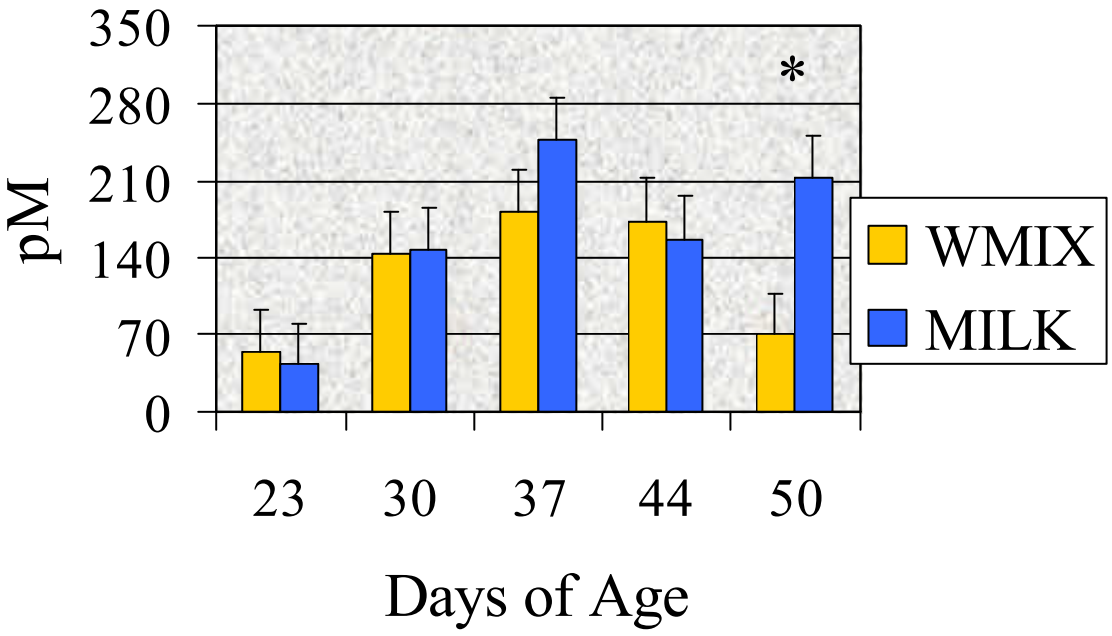


Figure 12. Plasma IGF-1.

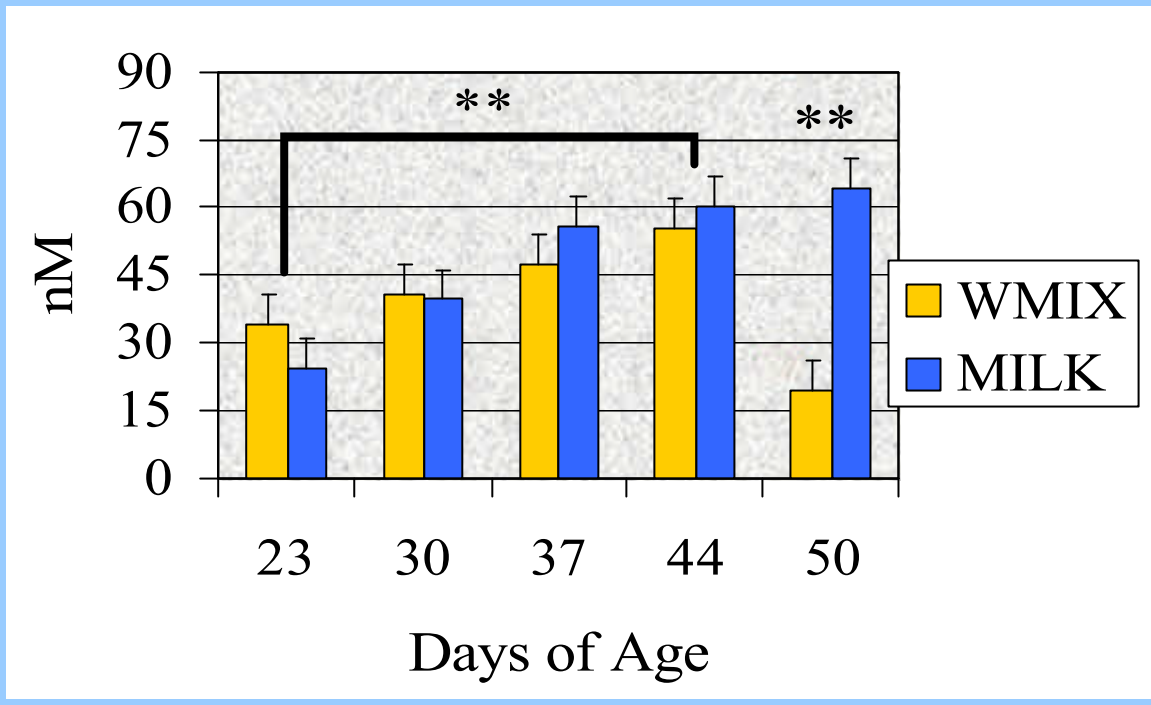
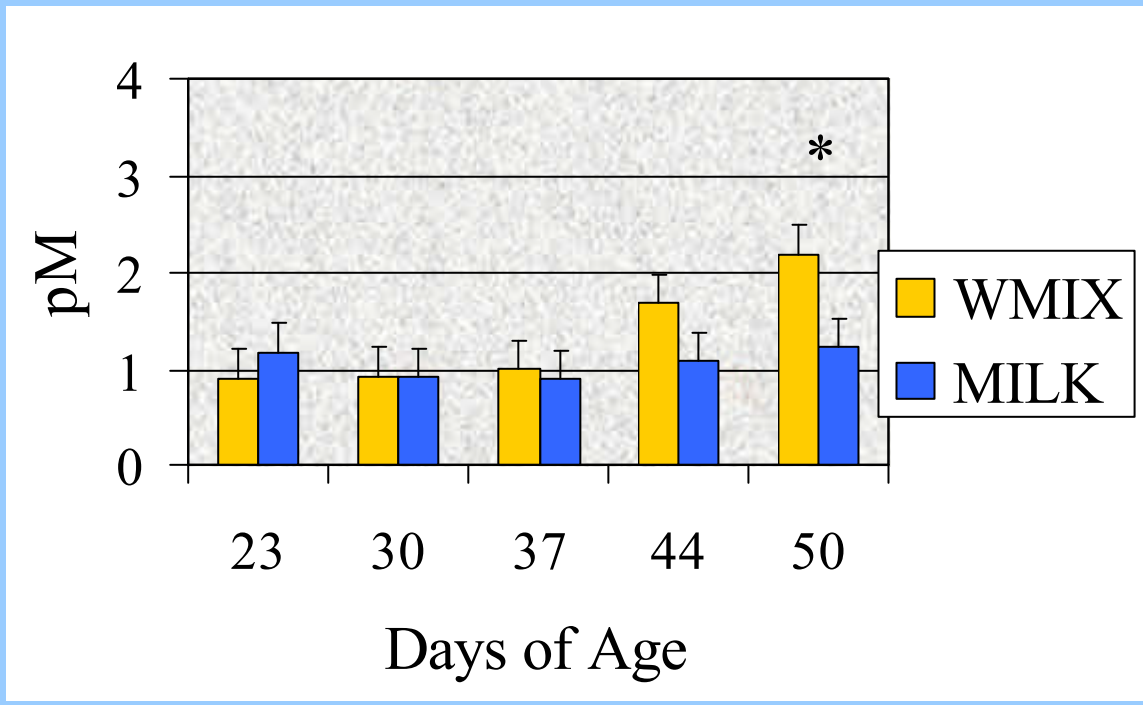


Figure 13. Plasma ghrelin.



3.6 Enzymatic Activity in Plasma

Results obtained from the analysis of plasmatic activity of ALT, AST and α-amylase are shown in figures 14, 15 and 16, respectively.

The activity of plasma ALT was significantly higher ($P<0.01$) in the WMIX group than the MILK one, during the last two weeks of life (figure 14).

Plasmatic activity of AST began to be higher ($P<0.05$) in the WMIX kids on day 44 of life. The difference was even greater ($P<0.01$), two days after the completion of weaning (figure 15).

No difference was observed in the activity of plasma α-amylase, between groups, during the whole study period (figure 16).

Figure 14. Plasmatic activity of ALT.

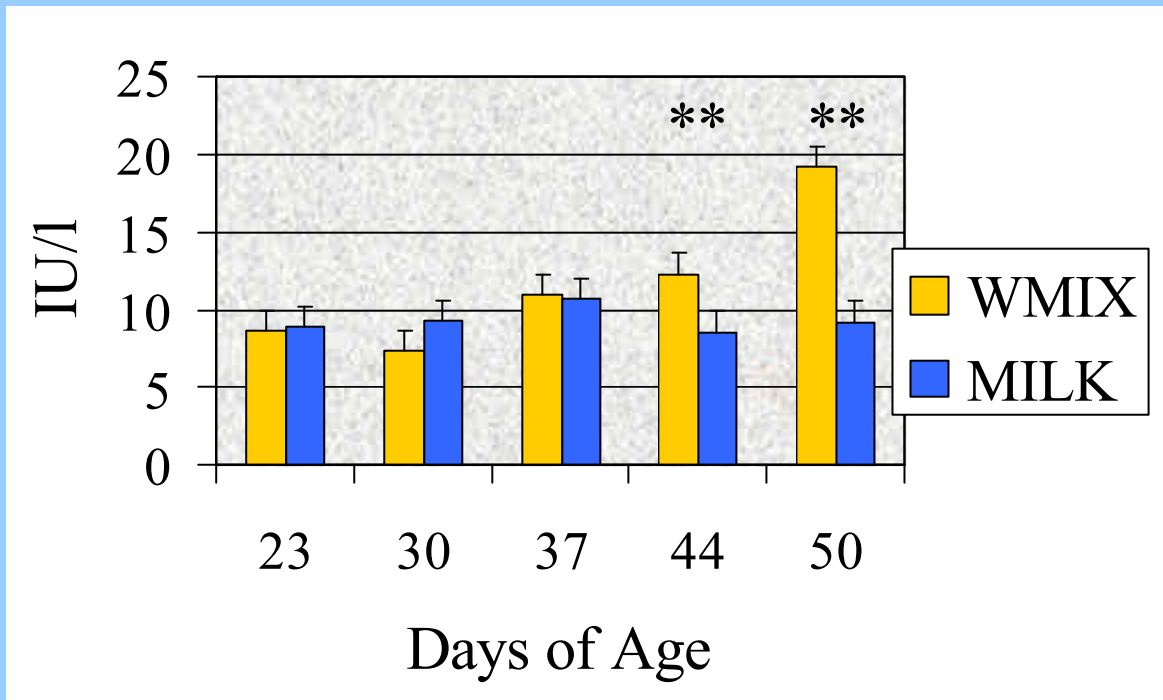


Figure 15. Plasmatic activity of AST.

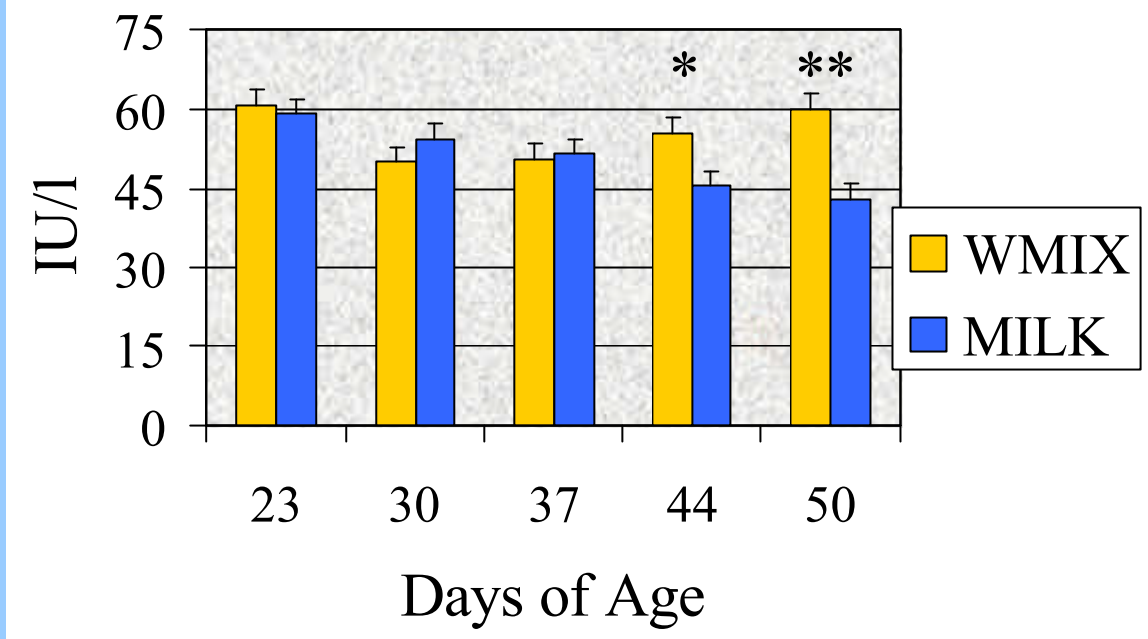
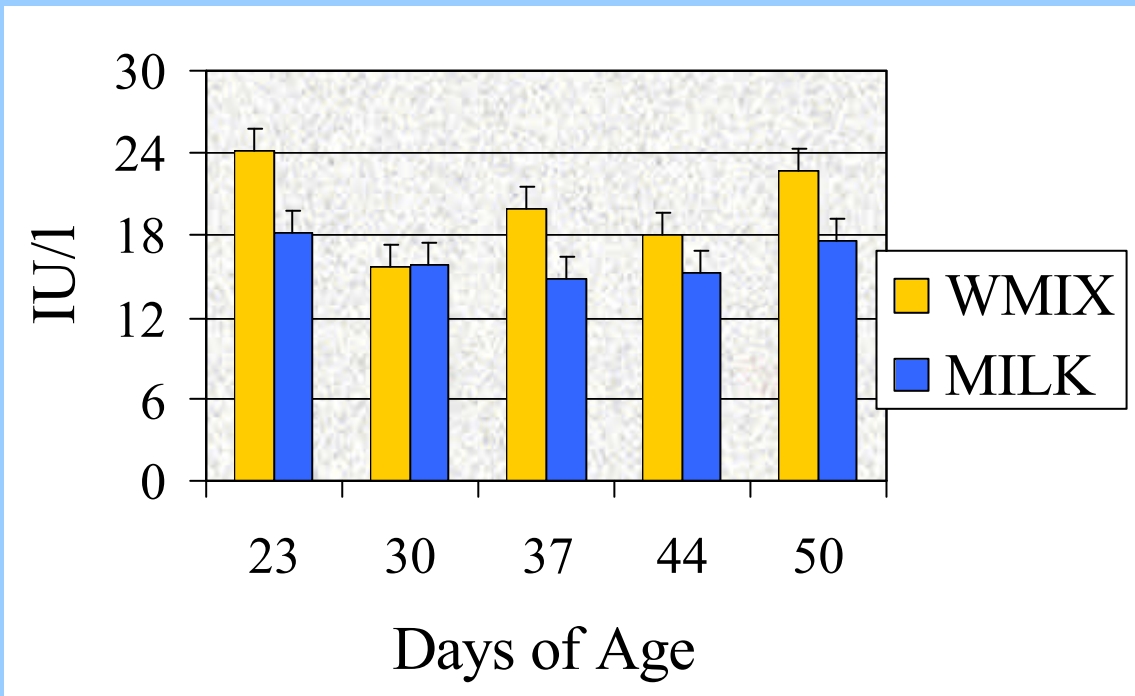


Figure 16. Plasmatic activity of α-amylase.



3.7 Haematic Parameters of Welfare

Data about cortisol and haptoglobin are presented in figure 17 and 18 respectively.

Plasma cortisol level began to be lower ($P<0.05$) in the WMIX group on day 44 of age. By contrast, no difference was observed in the concentration of plasma haptoglobin, during the entire study period.

Figure 17. Plasma cortisol.

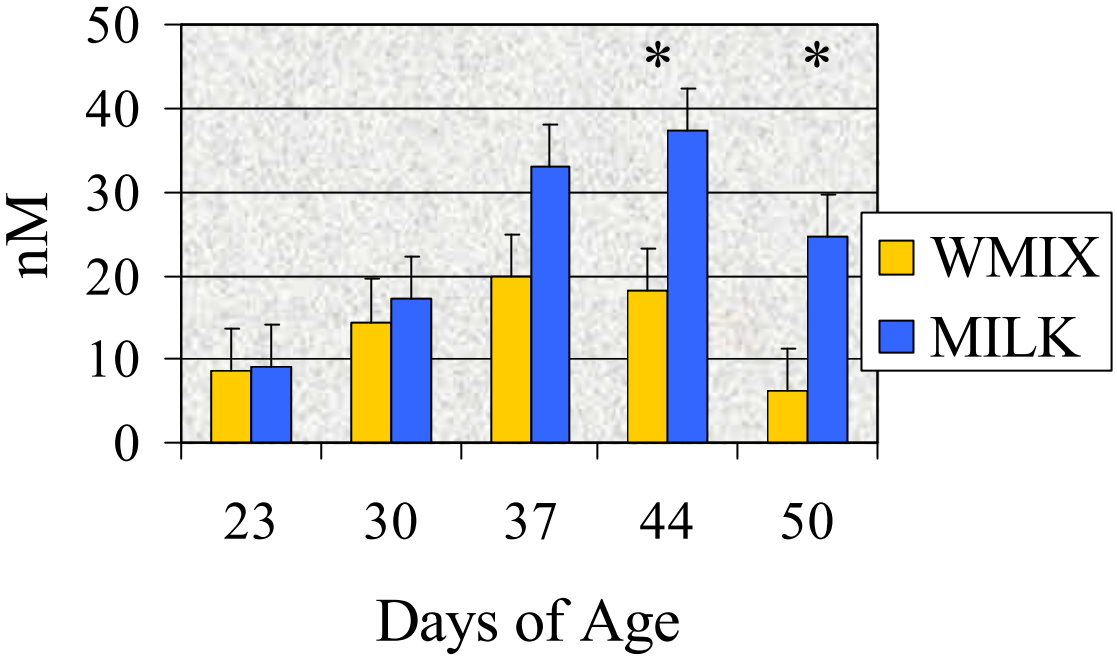
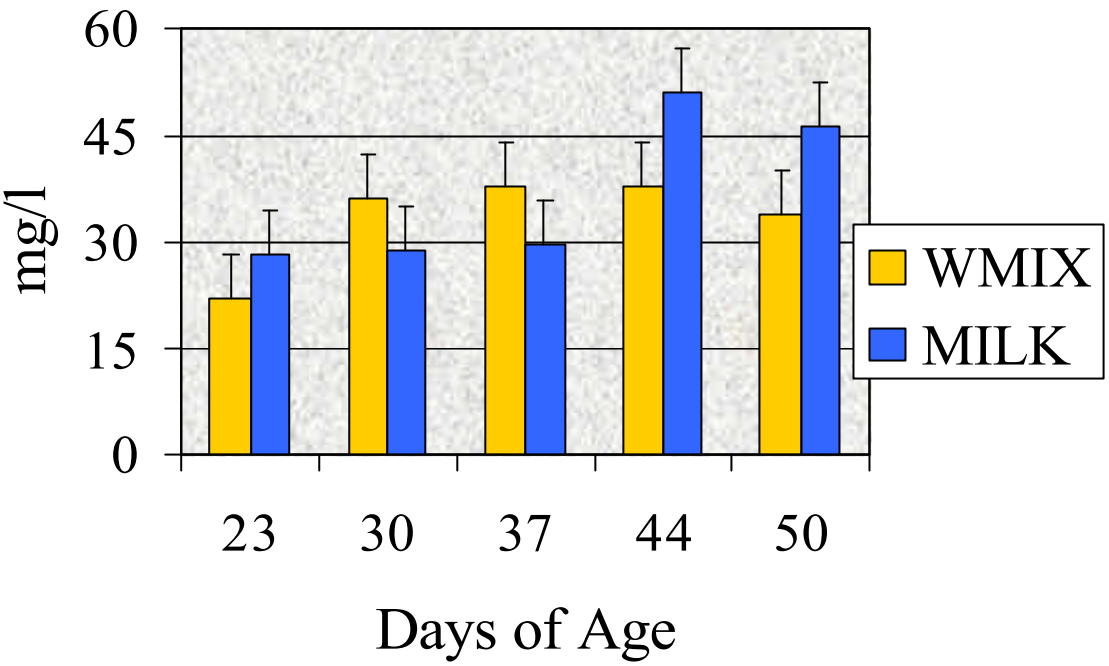


Figure 18. Plasma haptoglobin.



3.8 Slaughtering Parameters

Slaughtering parameters are presented in table 3.

Although BW, carcass and liver weights did not differ between the two groups, the MILK kids had a significantly higher ($P<0.05$) slaughtering yield than the WMIX ones (Table 3).

Table 3. Slaughtering parameters.

	WMIX	MILK	SEM
Live BW (kg)	15.3	16.2	1.56
Carcass Weight (kg)	9.54	10.7	0.93
Yield (%)	62.2*	66.2*	0.86
Liver Weight (g)	330	377	18.7

3.9 Liver and Pancreas Analysis

Results obtained from the analysis of liver and pancreas samples are shown in tables 4 and 5, respectively.

As observed in table 4, no difference was observed nor in liver DNA and RNA content, neither in their ratio, which is considered a measure of the synthetic potentiality of the liver (Munro and Flack, 1966). Similarly, no difference was observed in enzymatic activity of liver and phospholipids content, between the experimental groups. Also liver ribosomal capacity (RNA/soluble protein ratio) was not different between groups. However, epatic glycogen content was lower ($P<0.05$) in WMIX kids, compared to the MILK ones.

Table 4. Liver analysis. SP indicates soluble protein; P-lipids indicates phospholipids.

	WMIX	MILK	SEM
DNA (mg/g liver)	2.96	2.54	0.18
RNA (mg/g liver)	5.47	5.29	0,17
RNA/DNA	1.87	2.16	0.15
ALT (IU/g liver)	7.71	8.22	0.97
AST (IU/g liver)	200	229	17.9
ALT/DNA (IU/mg DNA)	2.63	3.50	0.53
AST/DNA (IU/mg DNA)	68.3	97.4	13.4
SP (mg/g liver)	223	238	8.90
ALT/SP (IU/mg SP)	34.2	34.5	2.29
AST/SP (IU/mg SP)	899	965	69.2
RNA/SP	0.025	0.022	0.002
Glicogen (mg/g liver)	40.9*	55.3*	4.77
P-lipids (mg/g liver)	3.80	3.87	0.12

As shown in table 5, pancreatic DNA and RNA content did not differ between the two groups, and there was no difference in RNA/DNA ratio which is an estimate of the extent of protein synthesis within each cell (Colombo et al., 2005). Pancreatic zymogen content did not differ either and there was no difference in RNA/zymogen (indicator of ribosomal capacity – Attaix et al., 1989) between the groups. By contrast, both pancreatic amylase activity expressed as international units (IU) per gram of fresh tissue and pancreatic amylase activity expressed as IU per mg of DNA were more than three times higher in the MILK than the WMIX group. Plasma insulin correlated strongly with pancreatic α-amylase activity expressed per gram of fresh tissue ($r=0.70$, $n=11$, $P<0.05$) and also with pancreatic α-amylase activity expressed per milligram of DNA ($r=0.67$, $n=11$, $P<0.05$). The correlation between insulin and amylase activity per mg of zymogen was even stronger ($r=0.85$, $n=11$ $P<0.01$).

Table 5. Pancreas analysis. Z indicates zymogen.

	WMIX	MILK	SEM
DNA (mg/g pancreas)	3.36	3.16	0.45
RNA (mg/g pancreas)	13.3	11.8	1.09
RNA/DNA	4.43	4.12	0.66
α-amylase (IU/g pancreas)	8.23*	29.5*	6.24
α-amylase (IU /mg DNA)	2.94*	8.87*	1.64
Z (mg/g pancreas)	10.8	9.65	1.17
α-amylase /Z (IU/mg Z)	0.70**	2.79**	0.50
RNA/Z	0.54	0.60	0.07

3.10 Literature Cited

Attaix D., Aurousseau E., Bayle G., Rosolowska-Huszcz D., Manghebati A. and Arnal M.: Influences of Age and Weaning on In Vivo Pancreatic Protein Synthesis in the Lamb. Journal of Nutrition, 1989; 119(3): 463-470.

Ewan R. C.: Predicting the Energy Utilization of Diets and Feed Ingredients by Pigs. In: Energy Metabolism, European Association of Animal Production Bulletin No. 43. Edited by van der Honing Y. and Close W. H. Published by Pudoc - Wageningen, Netherlands, 1989; pp. 271-274.

Munro H. N. and Fleck A.: The Determination of Nucleic Acids. Methods in Biochemistry Analalysis, 1966; 14: 113-176.

Sandrucci A., Rapetti L. and Galli A.: Stima del Valore Energetico del Latte di Capra. In: Atti del Convegno della Società Italiana delle Scienze Veterinarie, Salsomaggiore Terme, 27-30 settembre 1995, 49, 975-976.

4 DISCUSSION

4.1 Abnormal Behaviours

The absence of aggression, injurious behaviours and stereotypies, throughout the entire experimental period, suggests that all the animals were able to express normal behaviours, in accordance with the principles expressed by the "five freedoms" (FAWC, 2001).

4.2 Ingestion and Body Weights

DMI began to differ between the groups soon after WMIX were subjected to the weaning protocol based on the progressive substitution of milk with the solid feed (§ 2.3.3). The initial decrease could be due to refusal of the new diet by the WMIX animals. Subsequently, lower DMI in this group could be because products from ruminal activity were making an increasing contribution to the nutritional requirements of the animals. Nevertheless the groups did not differ in body weight, possibly because feed in the gut could have inflated WMIX body weight, particularly during the last week of life.

4.3 Metabolic Traits

In adult ruminants nutritional effect on metabolic traits is not so clear. In example, blood glucose level fluctuates minimally relative feed intake because its concentration is hormonally maintained within the physiological range by the interplay of the glucose-lowering action of insulin (Bharathi and Cryer, 2005) and the glucose-raising action of glucagon (Jiang and Zhang, 2003). Hyperglycemia can only develop when the rate of entry of glucose into plasma exceeds its rate of disposal. This occurs throughout the day in response to meals. However, this condition is quite transitory and within a short time euglycemia is restored (Chen et al., 1988). Anyway, in the present study, WMIX kids were subjected to a three-weeks period of profound changes in digestive function and activity and it is possible to suppose that all those changes have produced modifications in the basal levels of the investigated

parameters, that are more difficult to evidence in the adult animal, in which digestive functions are mature.

At two days post-weaning (age 50 days), plasma glucose, free amino acids and urea were lower in WMIX than MILK kids (§ 2.3.4). In adult ruminants, only starch that escapes from the rumen undergoes digestion by pancreatic enzymes, whereas plasma glucose is chiefly formed by gluconeogenesis from starch-derived propionate (Ortega-Cerrilla and Mendoza-Martinez, 2003) or from deamination of amino acids (Heitmann et al., 1973). As WMIX kids were in the early ruminant state, they may not have been able to use dietary starch efficiently, thereby explaining the low glucose and possibly also low free amino acids. In young lambs it is known that normal rumen maturation takes about 12 weeks (Baldwin, 2000). The lower glucose and free amino acids in WMIX kids could also have been due to the relative inability of the solid diet to supply sufficient protein and energy.

The lower plasma urea in the WMIX group may have been a consequence of urea recycling to the rumen. The lower N intake associated with the weaning diet may have stimulated urea recycling to the rumen, where it would have been used for microbial protein synthesis, thereby enhancing the efficiency of protein utilization (Ludden et al., 2003).

Furthermore, since only in the MILK group plasma urea was directly related to amino acid level ($r=0.49$, $n=30$, $P<0.01$), it is possible that animals of this group used dietary amino acids as energy source as well as to meet their anabolic needs. In non-ruminants and in the absence of kidney disease, amino acid transamination/deamination are the main factors influencing plasma urea level (Finco, 1989).

Plasma creatinine is considered an index of the muscle mass (Fekry et al., 1989) and increased levels may reflect increased protein catabolism (Finco, 1989). The higher levels of plasma creatinine in the WMIX group might therefore be the consequence of an initial tissue wasting, due to the change of the diet, not yet resulting in alteration in carcass weight or in plasma protein or albumin level.

4.4 Plasma Hormones

As observed in § 2.3.5, the differences in diet had no effect on plasma leptin at any time during the study period. Other researchers, in a recent experiment on lambs, have not observed differences in plasma leptin concentration between pre- and post-weaning lambs (Tokuda et al., 2003). Plasma leptin level of kids of both groups decreased, between day 30 and day

44 of life. This result is in accordance with other observations in humans. In 1998, in fact, Helland and coll. observed a reduction in plasma leptin level from birth to 4 weeks of age. After that, it was observed an increase in plasma leptin concentration from 4 to 14 weeks after birth (Helland et al., 1998). The experimental period of the present study was too shirt to detect an eventual arise in plasma leptin level, that in goats occurs at the onset of puberty (Vitali et al., 2005).

Contrarily to leptin, ghrelin level was similar in both groups during the most of the study period, but, two days after the completion of weaning, they were significantly higher in the WMIX group, compared to the MILK one (§ 2.3.5). In infants ghrelin exerts a strong growth hormone-releasing action primarily to promote body growth (Savino et al., 2006); in mammals it also acts on the central nervous system to stimulate food intake (Kojima and Kangawa, 2005). In sheep, circulating ghrelin peaks before the onset of a meal (Sugino et al., 2002) and in humans, plasma ghrelin level increases during fasting and decreases after food intake (Tschop et al., 2001). Obtained data induce to speculate that the higher plasma ghrelin level in the weaned animals could have been due to the lower DMI, which was particularly low at the completion of weaning. This hypothesis is in contrast with other recent findings. In fact, in 2006, Kobayashi and coll. observed that plasma ghrelin concentration in goats was not changed by feeding or weaning, suggesting that it has to be clarified whether the regulation of ghrelin secretion in goat kids is similar to that of other species (Kobayashi et al., 2006).

Plasma insulin was three times lower in the WMIX than MILK group two days after complete weaning (age 50 days) (§ 2.3.5). The difference may have been related to the lower plasma glucose and amino acids in the WMIX group at this time. In fact, plasma insulin level strongly correlated to glucose ($r=0.54$, $n=55$, $P<0.01$) and amino acids ($r=0.39$, $n=55$, $P<0.01$). Anyway, the possibility arises that the higher insulin in the MILK group could be due to insulin supplied with the milk. In 1998, Kinouchi and coll. found that plasma insulin increased in rats during early weaning, and that spiking of milk with physiological levels of insulin reinforced this increase. Insulin is naturally present in goat's milk at levels of about 272 pmol/l, in mid lactation (Magistrelli et al., 2005). On day 50, MILK kids were ingesting about 1000 pmol of insulin per head per day. Several milk-borne peptides can pass the gastro-intestinal mucosa (Gonnella et al., 1987) in a receptor-mediated process (Xu et al., 2000; Blum and Baumrucker, 2002) to enter the systemic circulation and supplement the suckling animal's production of these substances (Kinouchi et al., 2000). However it is unclear whether milk-borne insulin can cross the mucosa and enter the circulation in suckling kids in a long-lasting bioactive form.

Plasma level of IGF-1 constantly increased in both the experimental groups, between day 23 and day 44 of age, then rapidly decreased in the WMIX kids, during the last week of life (§ 2.3.5). Once again, the result could have been due to the lower DMI in WMIX animals, which was particularly low at the completion of weaning. In fact, IGF-1 is markedly dependent on nutritional status (Breier, 1999) and the main regulation of its plasma level is associated with feed intake, especially with amino acids and energy, respectively (Noguchi, 2000). In support to this idea, plasma IGF-1 strongly correlated to plasma glucose ($r=0.61$, $n=55$, $P<0.01$) and plasma amino acids ($r=0.60$, $n=55$, $P<0.01$), which were significantly lower in WMIX kids, two days after the completion of weaning. However, as done for insulin, it is not possible to exclude that the higher IGF-1 in the MILK group was due to IGF-1 consumption with the milk. Also IGF-1 is naturally present in goat's milk at levels of about 1.20 nmol/l, in mid lactation (Magistrelli et al., 2005). Moreover, both colostral and milk IGF-1 are supposed to transpass the gastro-intestinal mucosa via a receptor-mediated process (Blum and Baumrucker, 2002; Sparks et al., 2003). Insulin and IGF-1 share many properties. In particular, IGF-1 has an anabolic action consisting in stimulating the uptake of amino acids and glucose by the cells, with a mechanism similar, although much lower, to that of insulin (Noguchi, 2000). In the present study insulin and IGF-1 were strongly correlated ($r=0.65$, $n=55$, $P<0.01$).

4.5 Enzymatic Activity in Plasma

As observed in § 2.3.6, WMIX kids had higher levels of plasmatic activity of ALT and AST.

When certain types of cell are damaged, they can leak enzymes into the bloodstream. ALT and AST are examples of such enzymes. Their levels in plasma are markedly elevated in hepatitis or other acute liver damages (Teran et al., 1995). ALT and AST are members of the transaminase family of enzymes. ALT catalyzes the transfer of amino groups between alanine and glutamate to meet physiological needs. AST catalyzes the transfer of amino and keto groups between a-amino acids and a-keto acids. ALT and AST are also found in the heart, kidneys and in muscles (Tolman and Rej, 1999), so it is possible to hypothesize that the difference observed might therefore be the consequence of an initial tissue wasting, due to the change of the diet, as confirmed by the difference in creatinine level.

However, in case of tissue damage, ALT and AST activity in plasma usually have an higher and more rapid increase than that observed in the

present experiment (Teran et al., 1995; Tolman and Rej, 1999). One possible reason for the obtained result is that the higher ALT and AST activities in plasma of WMIX kids have been caused by the change of diet. In humans, in fact, the reduction of protein intake may increase the activity of transaminases in plasma (Garza et al., 1977).

4.6 Haematic Parameters of Welfare

MILK kids had higher level of plasma cortisol, in the last seven days of the study period (§ 2.3.7). Analysis of haematic cortisol is considered the most powerful parameter to asses stress (Brown-Borg et al., 1993; Bradshaw et al., 1996; Pol et al., 2002). However, MILK kids were maintained under the same conditions for the entire experimental period, so there is no reason to consider the MILK animals stressed, between day 44 and day 50 of age. The absence of difference in plasma haptoglobin level support this idea.

The correlation between plasma urea and amino acid levels in MILK kids ($r=0.49$, $n=30$, $P<0.01$) induces to hypothesize that animals of this group used a part of the dietary amino acids as energy source (§ 3.3). In this case, the arise in plasma cortisol could have stimulated the liver to convert amino acids to glucose for energy. Glucocorticoids, in fact, play an important role in gluconeogenesis from non-glucidic substrates (Matteri et al., 2000).

4.7 Slaughtering Parameters

MILK group kids had a higher slaughtering yield than WMIX group animals (§ 2.3.8), although no difference was observed in body, carcass and liver weight. This result could be a possible consequence of the higher DMI and greater efficiency of the MILK diet. Anyway, the live weight of WEAN kids could have been overestimated by the presence of feed in the rumen, particularly during the last week of life.

4.8 Liver and Pancreas Analysis

Liver was analyzed for DNA, RNA, soluble protein, glycogen and phospholipids content and ALT and AST activity. Pancreas was analyzed for DNA, RNA and zymogen content and α-amylase activity.

DNA level provides an index of the number of cells in a tissue and, in consequence, can be considered one basis on which other cell components can be expressed, while the concentration of RNA in a tissue can be correlated with the intensity of protein synthesis (Munro and Fleck, 1966).

As shown in § 2.3.9, liver analysis pointed out differences between weaned (WMIX) and unweaned (MILK) animals for epatic glycogen content, which was lower in WMIX group, as possible consequence of the lower level of plasma glucose in this group, respect the MILK one. Glycogen content of liver was correlated to plasma glucose ($r=0.55$, $n=11$, $P<0.05$).

No difference was observed in pancreatic DNA and RNA content (or RNA/DNA ratio) between the two groups, suggesting no difference in the synthetic potential of the organ. This suggestion is supported by the findings that no difference was observed neither in pancreatic zymogen content and RNA/zymogen ratio, which can be considered an estimate of the ribosomal capacity of pancreas (Attaix et al., 1989). However these inferences depend on the assumption that, in the early post-weaning days, pancreas weight in the WMIX group did not differ from that of milk fed kids of the same age (Attaix et al., 1989).

Although the plasma α-amylase activity and pancreatic zymogen content did not differ between the groups, at age 50 days, pancreatic amylase activities (per g pancreas, per mg pancreatic DNA and per mg of zymogen) were more than three times higher in the MILK than WMIX group (§ 2.3.9). This lower digestive enzyme activity could be due to weaning-induced stress due to an interaction between new dietary substrates and developing ruminal activity. However, in the present study stress was minimized by the gradual nature of the weaning process (lasting 18 days). Furthermore, the reduction in DMI at the beginning of weaning, with no significant stall in weight gain, suggests that the WMIX kids were successfully adapting to the new diet and that feed intake was adequate.

The finding that, in newly weaned goat kids, pancreatic α-amylase activity was lower than in MILK kids represent the intriguing result of the present study. The presence of starch in the diet of the WMIX animals did not therefore stimulate pancreatic α-amylase activity. The enzyme content of the pancreas is the net result of continuous synthesis and secretion, although enzyme content and enzyme activity are not always directly related (Swanson et al., 2000). Weaned lambs fed a high energy/high starch diet tend to have more pancreatic

α-amylase and greater amylase activity, while transcript levels tend to be lower, suggesting that transcription is inhibited (or mRNA stability reduced) by the diet with parallel inhibition of secretion, resulting in the accumulation of enzyme in pancreatic tissue (Swanson et al., 2000). In any event, lowered transcript levels suggest that dietary regulation of pancreatic α-amylase expression is complex in ruminants and probably mediated both at the transcriptional and posttranscriptional levels (Swanson et al., 2000).

It is possible that the absence of complex dietary carbohydrates could have inhibited the secretion of the enzyme into the duodenum of the MILK animals, while the synthetic activity of the pancreas was not impaired in newly weaned kids, resulting in a greater pancreatic storage. Our data on the RNA content and ribosomal capacity (RNA/zymogen) of the pancreas of weaned and milk-fed kids indicate similar synthetic potential and support this possibility. Furthermore, in eight-week old lambs, weaning does not alter pancreatic ribosomal capacity, compared to that in prolonged-suckling animals of the same age (Attaix et al., 1989).

Another possible explanation for the obtained result is that the lower quality or quantity of dietary protein received by the WMIX group around weaning could have limited the pancreatic content and activity of α-amylase, as previously documented (Harmon, 1992). This idea is supported by the finding that weaned kids had lower plasma amino acids and urea. On the other hand, the finding that the RNA content and ribosomal capacity of the pancreas of weaned and milk-fed kids did not differ militates against this hypothesis.

Another possible reason for the greater α-amylase activity in milk-fed pre-ruminant kids is that it was stimulated by the higher glucose or insulin levels, as occurs in monogastric mammals (Brannon, 1990; Kinouchi et al., 1998). While in beef steers pancreatic secretion of α-amylase is not directly related to plasma glucose and insulin (Walker and Harmon, 1998), in rats there is a close relationship between the increased plasma insulin just before weaning, and pancreatic α-amylase activity, suggesting that insulin may stimulate pancreatic functional development (Kinouchi et al., 1998; Kinouchi et al., 2000). In support of this there is also the correlations founded between plasma insulin and pancreatic α-amylase activity expressed per gram of fresh tissue ($r=0.70$, $n=11$, $P<0.05$) and between insulin and pancreatic α-amylase activity expressed per milligram of DNA ($r=0.67$, $n=11$, $P<0.05$). The correlation between insulin and amylase activity per mg of zymogen was even stronger ($r=0.85$, $n=11$, $P<0.01$).

4.9 Literature Cited

Attaix D., Aurousseau E., Bayle G., Rosolowska-Huszcz D., Manghebati A. and Arnal M.: Influences of Age and Weaning on In Vivo Pancreatic Protein Synthesis in the Lamb. Journal of Nutrition, 1989; 119(3): 463-470.

Baldwin R. L.: Sheep Gastrointestinal Development in Response to Different Dietary Treatments. Small Ruminant Research, 2000; 35: 39-47.

Bharathi R. and Cryer P. E.: Maintenance of the Postabsorptive Plasma Glucose Concentration: Insulin or Insulin plus Glucagon? American Journal Physiology: Endocrinology and Metabolism, 2005; 289: E181-E186.

Blum J. W. and Baumrucker C. R.: Colostral and Milk Insulin-like Growth Factors and Related Substances: Mammary Gland and Neonatal (Intestinal and Systemic) Targets. Domestic Animal Endocrinology, 2002; 23: 101-110.

Bradshaw R. H., Parrott R. F., Goode J. A., Lloyd D.M., Rodway R. G. and Broom D. M.: Behavioural and Hormonal Responses of Pigs During Transport: Effect of Mixing and Duration of Journey. Animal Science, 1996; 62: 547-554.

Brannon P. M.: Adaptation of the Exocrine Pancreas to Diet. Annual Reviews in Nutrition, 1990; 10: 85-105.

Breier B. H.: Regulation of Protein and Energy Metabolism by the Somatotropic Axis. Domestic Animal Endocrinology, 1999; 17: 209-218.

Brown-Borg H. M., Klemcke, H. C. and Blecha, F.: Lymphocyte Proliferative Responses in Neonatal Pigs With High or Low Plasma Cortisol Concentration After Stress Induced by Restraint. American Journal of Veterinary Research, 1993; 54: 2015-2020.

Chen Y. D. I., Jeng C. Y., Hollenbeck C. B., Wu M. S. and Reaven G. M.: Relationship Between Plasma Glucose and Insulin Concentration, Glucose Production, and Glucose Disposal in Normal Subjects and Patients with

Non-Insulin-dependent Diabetes. Journal of Clinical Investigation, 1988; 82: 21-25.

FAWC: Interim Report on the Animal Welfare Implications of Farm Assurance Schemes. Published by The Farm Animal Welfare Council - London, UK; 2001.

Fekry A., Abdelaa A., Shebaita M. and Salem M.: Is Creatinine a Good Indicator for Meat Production in Fat-tailed Sheep? In: Ruminant Production in Dry Subtropics: Constraints and Potential, EAAP Publication No. 38, 1989: 150-152.

Finco D. R.: Kidney Function. In: Clinical Biochemsitry of Domestic Animals, 4th Edition. Edited by Kaneko J. J., Harvey J. W. and Bruss M. L. Published by Academic Press - San Diego, CA, USA, 1989, pp. 496-542.

Garza C., Scrimshaw N. S. and Young A. R.: Human Protein Requirements: A Long-term Metabolic Nitrogen Balance Study in Young Men to Evaluate the 1973 FAO/WHO Safe Level of Egg Protein Intake. Journal of Nutrition, 1977; 107: 335-352.

Gonnella P. A., Siminoski K., Murphy R. A. and Neutra M. R.: Transepithelial Transport of Epidermal Growth Factor by Absorptive Cells in Suckling Rat Ileum. Journal of Clinical Investigation, 1987; 80: 22-32.

Harmon D. L.: Impact of Nutrition on Pancreatic Exocrine and Endocrine Secretion in Ruminants: a Review. Journal of Animal Science, 1992; 70: 1290-1301.

Heitmann R.N., Hoover W.H. and Sniffen J.: Gluconeogenesis from Amino Acids in Mature Wether Sheep. Journal of Nutrition, 1973; 103: 1587-1593.

Helland I. B., Reseland J. E., Saugstad O. D. and Drevon C. A.: Leptin Levels in Pregnant Women and Newborn Infants: Gender Differences and Reduction During the Neonatal Period. Pediatrics, 1998; 101(1): 12-16.

Jiang G. and Zhang B. B.: Glucagon and Regulation of Glucose Metabolism. American Journal of Physiology: Endocrinology and Metabolism, 2003; 284: E671–E678.

Kinouchi T., Koizumi K., Kuwata T. and Yajima T.: Crucial Role of Milk-borne Insulin in the Development of Pancreatic Amylase at the Onset of Weaning in Rats. American Journal of Physiology: Endocrinology and Metabolism, 1998; 275: 1958-1967.

Kinouchi T., Koizumi K., Kuwata T. and Yajima T.: Milk-borne Insulin With Trypsin Inhibitor in Milk Induces Pancreatic Amylase Development at the Onset of Weaning in Rats. Journal of Pediatric Gastroenterology and Nutrition, 2000; 30(5): 515-521.

Kobayashi Y., Onodera M., Yoshida M., Sugino T., Katoh K. and Obara Y.: Postprandial Changes in Plasma Concentrations of Growth Hormone and Ghrelin Around Weaning in the Goat. General Compound in Endocrinology, 2006; 148: 368-374.

Kojima M and Kangawa K.: Ghrelin: Structure and Function. Physiological Reviews, 2005; 85: 495-522.

Ludden P. A., Wechter T. L., Scholljegerdes E. J. and Hess B. W.: Effects of Oscillating Dietary Protein on Growth, Efficiency, and Serum Metabolites in Growing Beef Steers. Professional Animal Science, 2003; 1930-1934.

Magistrelli D., Valli A. and Rosi F.: Insulin and IGF-1 in Goat Milk: Influence of the Diet. Italian Journal of Animal Science, 2005; 4 (Supp. 2): 386-388. (Proceeding of the ASPA XVI Congress, Torino, June 28-30, 2005).

Matteri R. L., Carroll J. A. and C.J.. Dyer C. J.: Neuroendocrine Responses to Stress. In: The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Edited by Moberg G. P. and Mench J. A. Published by CAB International; 2000: pp. 43-76.

Munro H. N. and Fleck A.: The Determination of Nucleic Acids. Methods in Biochemistry Analalysis, 1966; 14: 113-176.

Noguchi T.: Protein Nutrition and Insulin-like Growth Factor System. British Journal of Nutrition, 2000; 84(Suppl. 2): S241-S244.

Ortega-Cerrilla M. E. and Mendoza-Martinez G.: Starch Digestion and Glucose Metabolism in the Ruminant: a Review. Interciencia, 2003; 28: 380-386.

Pol F., Courboulay V., Cotte J. P., Martrenchar A., Hay M. and Mormède P.: Urinary Cortisol as an Additional Tool to Assess the Welfare of Pregnant Sows Kept in Two Types of Housing. Veterinary Research, 2002; 33: 13-22.

Savino F., Grassino E. C., Fissore M. F., Guidi C., Liguori S. A., Silvestro L., Oggero R. and Miniero R.: Ghrelin, Motilin, Insulin Concentration in Healthy Infants in the First Months of Life: Relation to Fasting Time and Anthropometry. Clinical Endocrinology, 2006; 65: 158-162.

Sparks A. L., Kirkpatrick J. G., Chamberlain C. S., Waldner D. and Spicer L. J. : Insulin-like Growth Factor-I and its Binding Proteins in Colostrum Compared to Measures in Serum of Holstein Neonates. Journal of Dairy Science, 2003; 86: 2022-2029.

Sugino T., Hasegawa Y., Kikkawa Y., Yamaura J., Yamagishi M., Kurose Y., Kojima M., Kangawa K. and Terashima Y.: A Transient Ghrelin Surge Occurs Just Before Feeding in Scheduled Meal-fed Sheep. Biochemical and Biophysical Research Communication, 2002; 295: 255-260.

Swanson K. C., Matthews J. C., Matthews A. D., Howell J. A., Richards C. J. and Harmon D. L.: Dietary Carbohydrate Source and Energy Intake Influence the Expression of Pancreatic α-amylase in Lambs. Journal of Nutrition, 2000; 130: 2157-2165.

Teran J. C., Bashour F. N., Sondhi S. S. and Mullen K. D.: Validation of a Discriminant Score for the Diagnosis of Cirrhosis in Patients With Chronic Hepatitis. Gastroenterology, 1995; 108: A1185-A1194.

Tokuda T., Delavaud C. and Chilliard Y.: Plasma Leptin Concentration in Pre- and Post-weaning Lambs. Animal Science, 2003; 76: 221-227.

Tolman K. G. and Rej R.: Liver Function. In: Tietz Textbook of Clinical Chemistry. Edited by: Burtis C. A. and Ashwood E. R. Published by: W. B. Saunders Company - Philadelphia, PA, USA, 1999; pp. 1125-1177.

Tschop M., Wawarta R., Riepl R. L., Friedrich S., Bidlingmaier M., Landgraf R. and Folwaczny C.: Post-prandial Decrease of Circulating Human Ghrelin Levels. Journal of Endocrinological Investigation, 2001; 24: RC19-RC21.

Vitali A., Magistrelli D., Rosi F., Azevedo J., Bernabucci U. and Ronchi B.: Leptin and the Onset of Puberty in Goat. Italian Journal of Animal Science, 2005, 4(Suppl. 2): 383-385. (Proceeding of the ASPA XVI Congress, Torino, June 28-30, 2005).

Walker J. A. and Harmon D. L.: Influence of Ruminal or Abomasal Starch Hydrolysate Infusion on Pancreatic Exocrine Secretion and Blood Glucose and Insulin Concentrations in Steers. Journal of Animal Science, 1998; 73: 3766-3774.

Xu R. J., Wang F. and Zhang S. H.: Postnatal Adaptation of the Gastrointestinal Tract in Neonatal Pigs: a Possible Role of Milk-borne Growth Factors. Livestock Production Science, 2000; 66: 95-107.

5 CONCLUSIONS

In January 2004, Ireland took over the presidency of the European Union. The Irish Society for the Prevention of Cruelty to Animals presented a memorandum laying down the animal welfare priorities for the next six months. Safeguarding animal health and welfare became a key viewpoint for the European Union and its importance also improved during the second half of the year, when Netherlands held the presidency.

Since then, animal welfare began to be seen as a trade opportunity for improving the quality of animal products.

As demonstrated in the present experiment, good management and husbandry and adequate nutrition form the basis of disease prevention and good animal welfare. In particular, the adoption of a gradual weaning process, lasting 18 days and consisting of a progressive substitution of milk with a solid diet, may help to minimize the stress of the transition from pre-ruminant to ruminant state, which represent the most dramatic event in the life of young ruminants.

Moreover, the livestock practice adopted in the present study permitted the normal development of the animal used, avoiding growth stasis.

Finally, attention is pointed out on the main findings of the present study: in goat kids undergoing weaning the introduction of starch to the diet greatly reduces pancreatic a-amylase activity. Plasma glucose, insulin and IGF-1 are also lower in weaned goat kids, compared to levels in milk-fed animals of the same age.

It is therefore possible that milk-borne peptides, such as insulin and IGF-1, transpass the gastro-intestinal mucosa, via a receptor-mediated process and enter the systemic circulation of the suckling kids. Moreover, milk-borne insulin seems to play a role in the development of pancreatic amylase synthesis and activity. Further studies are required to determine whether milk-borne insulin passes the gastro-intestinal mucosa of suckling kids and contributes to the functional development of the pancreas.

ACKNOWLEDGEMENTS

Special thanks to Professor Fabia Rosi – who made the experiment possible – for all the teachings and for the help in the analysis of plasma and tissual parameters.

Thanks to Professor Luca Rapetti for the formulation of the weaning mixture and for feeds analysis.

Thanks to Dr. Giorgia Polo Dimel for the help in tissue sampling, in the analysis of plasma metabolites and the help in livestock practices. For the same reason and for the help in blood and feeds sampling, special thanks also to Dr. Paolo Roveda, Mr. Davide De Angeli, Mr. Giuseppe Scichili, Mr. Paolo Binda and Mr. Marco Misitano.

Finally, special thanks to my friends, my parents, Anna and Adriano, and my wife, Veronica for the support and the help in the drawing up of the present work.

Damiano Magistrelli