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FEEDING A FREE CHOICE CAFETERIA-STYLE MINERAL VITAMIN SUPPLEMENT TO TRANSITION COWS: IMPACT ON SHORT TERM HEALTH AND PERFORMANCES

Somministrazione di un supplemento minerale-vitaminico in stile caffetteria alle bovine da latte in transizione: impatto sul benessere sul breve periodo e sulle performance

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ABSTRACT

The transition period is a critical phase during the lactating cows' life. In fact, it is characterized by many factors like for example decreased intake, negative energy balance, lipomobilization and increased calcium requirements, which may concur to the incidence of several diseases like ketosis, mastitis, hypocalcaemia, retained placenta and fatty liver. The supplementation with sugar–mineral-vitamin products during the dry and transition period may help in satisfying all the requirements of the cows. The aims of the present work were to evaluate the use of a free choice energetic mineral-vitamin supplement as a tool for the nutritional management of dry and transition cows and to study its effect on short term health and early lactation performances. A randomized complete block design was performed on 27 Italian Holstein cows divided into 3 groups subjected to 3 supplementation protocols: Control (CON), fed complete total mixed rations (TMR) specific for dry and lactating cows, covering the 75% of the mineral-vitamin requirements; the remaining 25% were administered as a top dressing to the control animals. The cows of the treated groups received respectively: Trockensteher® during the dry period, and Vitalyx® during lactating period, available *ad libitum* in the paddock (TV group); Trockensteher LIN/MOS® during the dry period, and Vitalyx® during lactating period, available *ad libitum* in the paddock (LV group). Groups intake, health status, metabolic parameters, time spent feeding in the manger and time spent ruminating, as well as the productive performances were evaluated from drying off to 20 days in milk (DIM). The TMR was a hay-based diet with prevalence of grass hay in the dry diet, and prevalence of alfalfa hay in the lactating diet. The TV group consumed less product during the dry period compared to the LV group, demonstrating also the lowest TMR intake in the dry period while an opposite trend was observed during the lactating period, in association also with a lower fecal score at 20 DIM. Both the tested protocols tendentially induced a higher time spent ruminating per kg of milk. Starting from a similar body condition score (BCS) at -60 DIM, the CON group showed a higher BCS at calving, with an increased *post-partum* lipomobilization with a raised probability of the occurrence of metabolic diseases. In fact, BHBA and NEFA plasma content at -7 DIM were higher in this group and plasmatic urea was decreased at 6 DIM. Both transition supplementation protocols resulted in a lowered milk production during the first 2 weeks of lactation but reached a similar milk yield in comparison to the CON group at 20 DIM. The

ad libitum supplementation with a mineral-vitamin blocks has proved to be a good alternative to help the metabolism of lactating cows during the critical transition period.

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CHAPTER 1: General introduction

THE TRANSITION DAIRY COW

The transition period of dairy cows is described by Grummer, 1995) as the period from 3 weeks before calving to 3 weeks after calving. During this period, the animal undergoes nutritional, metabolic and physiological modifications that predispose it to an increase in metabolic disorders and a greater onset of pathologies (Mulligan and Doherty, 2008). The most frequent metabolic pathologies recognized in this phase are hypocalcaemia, ketosis, fatty liver disease, metritis, mastitis and placental retention. In recent years, more and more importance has been given to the transition cow and this has led to an increase of studies and research with the aim of learning better its biology and nutritional requirements, ascertaining correct management strategies for this delicate (Redfern et al., 2021a). Currently, the management of the transition cow considers a few key factors which are mainly based on herding strategies and feeding programs (van Saun and Sniffen, 2014). These management practices are suggested to reduce the incidence of disease and minimize the influence of social stress by reducing the movement of animals in alternative groups and providing animals with a spacious, clean and comfortable environment (Redfern et al., 2021b). Nutrition also plays a fundamental role in maintaining the health of the transition cow, in fact diets in this delicate phase should ensure an optimal balance of minerals, micronutrients, and energy and protein (Lean et al., 2013). Management practices include not only grouping and feeding techniques but also other key factors such as: rumen health, BCS control and udder hygiene. In particular, a correct control of the BCS in the animals helps to reduce immunosuppression, minimizing the mobilization of lipids and satisfying the demand for amino acids, energy and proteins (Redfern et al., 2021a).

GLUCIDIC AND LIPIDIC METABOLISM

The nutrient requirements of a transition cow due to a pregnant uterus at 250 days of gestation and necessities to the mammary gland at the start of lactation leads to a tripling of the demand for glucose, a doubling of the demand for aminoacids (AA) and increasing about five times the demand for fatty acids in this timeframe. To allow these changes to occur, the cow has homeorhetic controls in the nutrients partitioning. The homeorhetic adaptation of glucose metabolism to lactation consists in the concomitant increase in hepatic gluconeogenesis and the formation of glucose oxidation by peripheral tissues. The glucose thus formed will be used in the synthesis of lactose by the mammary gland. Propionate derived from rumen fermentation, lactate from the Cori cycle, AA from protein catabolism or visceral absorption and glycerol released during lipolysis in adipose tissue are the main substrates for hepatic gluconeogenesis in ruminants. The greatest contribution is that given by the propionate which reaches 60% during the transition period (Overton and Waldron, 2004).

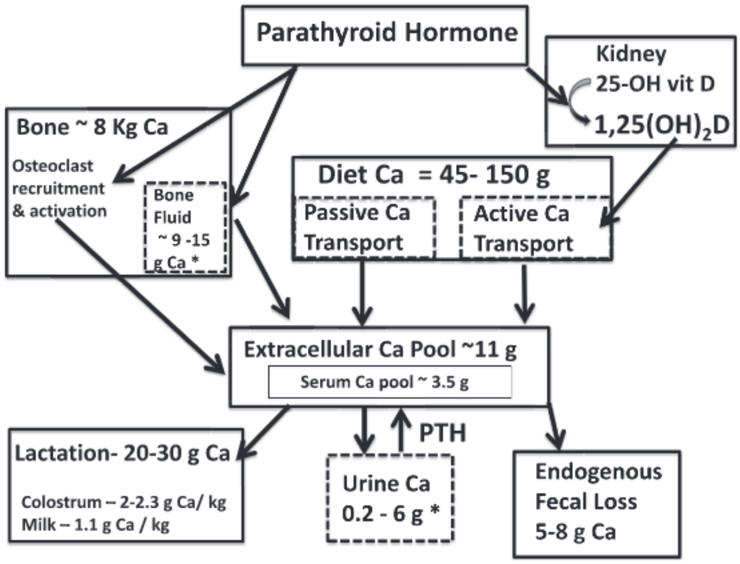
Contemporary, to satisfy the energy demand during the period of negative energy balance in early lactation, occur a massive mobilization of body fat stores in to the bloodstream in the form of Not Esterified Fatty Acids (NEFA). The 40% of milk fat during the first days of lactation depends on NEFA utilization, such as skeletal muscle uses some NEFA for fuel, decreasing its reliance on glucose as energy supplier during early lactation. Even if the liver takes up NEFA in proportion to their supply, typically it does not have sufficient capacity to metabolism them through export into the blood or catabolism for energy; in fact, cows are predisposed to accumulate NEFA as triglycerides within liver when large amounts are released from adipose tissue into the circulation. When liver NEFA accumulation occurs, some studies reported a decreased activity of liver in gluconeogenic capacity from propionate and a peripheral doubled concentrations of ammonia because of a decreased ureagenic capacity (Weiss, 1998) (McFadden, 2020).

For a correct management of the transition cow a focus on the influence of feeding level, feed ingredients and their interaction with BCS and liver function is needed. DMI and plasma NEFA concentrations are usually inversely related, and is reported a positive association between high pre-calving plasma NEFA and the incidence of metabolic disease. DMI in periparturient cows is reported to decline during the weeks preceding parturition despite an increase in energy requirements for fetal growth and lactogenesis. In particular cows fed DM and/or energy in excess of their requirements in dry period displayed the universally accepted decline in DMI before calving. Despite, cows fed either a high fibre ration or offered a restricted allowance of standard total mixed ration (TMR) to reduce energy intake *pre-partum* did not decline in DMI in the weeks pre-calving. Even if a slight negative energy balance (NEB) pre-calving may improve post-calving energy balance (EB), metabolic health indices and milk production, is important not to have an excessively decrease in DMI intake in *pre-partum* in order to not have an excess in NEFA mobilisation which can destabilize metabolic capacity of the liver.

CALCIUM HOMEOSTASIS

The Ca level in blood is kept between 2.1 and 2.5 mmol/L. About 50% is bound to proteins such as albumins, while the 42% and 48% exists in ionized form i.e., biologically active part. This ionized form can be influenced by metabolic conditions: in acid conditions it reaches close to 48% while in alkaline conditions close to 42%. In conditions of hypoalbuminemia, it is possible to have a low level of total Ca in the blood but relatively normal of ionized calcium, this happens at the time of calving. Before parturition, the calcium demands dramatically increase as it is sequestered by the mammary gland for colostrum formation. Calcium homeostasis as shown in Figure 1 is regulated by the parathyroid glands, which are highly sensitive to drops in calcium blood levels, reacting through the secretion of parathyroid hormone (PTH) (Goff, 2014).

Figure 1. Ca metabolism in dairy cow of 600 kg of body weight at the beginning of lactation (Goff, 2014) .



The primary targets of PTH are renal tubular epithelial cells, osteoclast and bone osteocytes, all of which express the PTH receptor. The first action of the hormone is exerted in the kidney where it promotes the reabsorption of Ca from proximal renal tubular fluids and also stimulates the synthesis of 1,25-dihydroxyvitamin D (1,25 (OH)2 D). If the changes in blood calcium levels are minimal, a renal stimulation may be enough to restore the level of calcaemia. At the beginning of lactation, the dairy cow loses 20 to 30g of calcium every day for the production of colostrum and early milk, so it has to mobilize calcium from the bones and increase its absorption by the intestine. Most of the Ca located in bone (> 99%) is found in hydroxyhepatite crystals linked to the collagen matrix and can only be mobilized by osteoclasts. Osteoclasts are indirectly activated by PTH, enhancing the cells activity producing enzymes and acid to digest bones matrix, in order to release Ca and P stored in form of calcium phosphate $Ca_3(PO_4)_2$ (Goff, 2014).

Another strategy to counteract hypocalcemia is through the absorption of by the intestine. The digestion of the inorganic Ca introduced through the diet takes place thanks to the acids contained in the abomasum, however much of the calcium is present in organic form in forage diet, therefore it is not available. The absorption mechanism take place under the stimulation of epithelial cells from the hormonal form of vitamin D, (1,25-dihydroxyvitamin D (1,25 [OH] 2D) stimulated by PTH. Vitamin D is synthesized by the skin following irradiation with the UV rays of the sun or is provided by the diet; in any case it is activated by the liver through a hydroxylation process forming 25-OH vitamin D, that travels to the kidney and is converted into 1,25 (OH) 2D. It reaches the intestine by binding to the intracellular vitamin D receptors (VDR), activating a series of vitamin D-dependent proteins that will allow calcium to be recovered. There is also a mechanism independent from vitamin D and allows the movement of calcium from the intestinal lumen to the extracellular fluid through the intestinal epithelium.

Also, other minerals have an important role in calcium homeostasis. Hypomagnesemia can disturb the hypocalcaemia regulation in two ways: reducing PTH secretion and the tissue sensitivity to PTH; hyperphosphatemia on the other hand acting on the production of 1,25-dihydroxyvitamin D altered the Ca homeostasis reducing the intestinal absorption and elevating the risk of hypocalcaemia; potassium interferes with the absorption of magnesium in the rumen and, as magnesium is important for calcium absorption, thereby increases the risk of milk fever (Goff, 2014).

PATOLOGIES AND METABOLIC DISEASE IN THE TRANSITION COW

Many farmers faced problems every year that are both inconvenient and frustrating. Not only clinical cases are part of the problem, indeed when clinical cases are evident far more cows suffer from subclinical conditions meanwhile. Most metabolic diseases occur during the transition period and, although genetic factors also influence the risk of these diseases, a proper management of animals in the weeks before and after parturition have a major effect against the onset of disease. The two most common metabolic disease encountered nowadays are milk fever and ketosis, although fatty liver and left displaced abomasum become even more common with greater supplement use and higher milk production of intensive system. All of these diseases have side effects which predispose cows to infections as a result of depression of the immune system which can affect particularly uterus and udder, reducing milk production and fertility.

KETOSIS

Ketosis is a common metabolic disease that affecting the transition dairy cattle, especially in the days after calving. A poor adaptive response to negative energy balance and a rapid lipolysis with an overproduction of NEFA leads to an accumulation of ketone bodies: BHBA, acetoacetate, and acetone. Symptoms of clinical ketosis include: reduced milk production, weight loss, dull coat, acetone smell of breath, fever and nervous sign like excess salivation or licking. Today, however, the major focus has shifted from clinical ketosis to the implications of subclinical ketosis (as defined by circulating BHBA >1.2 mmol/L). In dairy cows, the diagnosis of ketosis must be made as soon as possible in order to avoid the onset of other related diseases. Indeed, a greater risk of subsequent left displaced abomasum and metritis have been detected in animal suffering of subclinical ketosis. It was also observed an increased risk for the animal when BCS at calving increases from 3.25 to 3.50 (Edmonson et al. 1989), in fact the cows should be fed in order to reach a BCS of 3.0 at the moment of calving. The consumption of poor-quality silage which undergoes on a secondary fermentation is one of the risk factors that can lead to this pathology due to an increase of

BHBA in blood. For this reason, this kind of silage should possibly not be fed to transition dairy cows. The peak of BHBA in the animal during the post-partum period is strongly influenced by the diet before valving and in particular by the energy intake. It has been shown that cows which be fed with low-anergy diets before calving have lower plasma BHBA in the post-partum; in fact, excessive nutrition in the *pre-partum* period increases the risk of high BHBA in the blood after calving. Moreover, it was demonstrated that cows overfed before calving also showed molecular changes in the liver with reduced function of b-oxidation, gluconeogenesis and ureagenesis in the post-partum period (Overton and Waldron, 2004).

FATTY LIVER DISEASE

Fatty liver disease is a bovine metabolic disorder that can frequently occur during the transition period. It is characterised by reduced health status, lower productivity and defective reproductive performance. "Fat cow syndrome" is commonly related to fatty liver disease: it is a critical problem in cows that are over conditioned at parturition and results in dramatic decreases in DMI. Also, the rapid increase in milk yield, concomitant with physiological changes during the early *post-partum* period, concur at NEB and increased blood levels of NEFA. In the liver these molecules are re-esterified to triacylglycerol (TAG), stored in cytosol of hepatocytes or assembled with ApoB100, cholesterol and phospholipids to synthesise VLDL. Because of the high NEFA metabolism to overcome the synthesis of VLDL, during the first few weeks' *post-partum* occur a consistent increase in accumulation of TAG in the liver (Overton and Waldron, 2004).

It has been confirmed by several studies that hepatic lipidosis affects a large number of metabolic pathways, especially gluconeogenesis and ureagenesis, which often decrease. A consistent response evident in cows with moderate to severe fatty liver due to under-nutrition is the reduction in expression of genes and proteins associated with cholesterol synthesis, ATP production, endogenous carnitine synthesis and FA desaturation. In contrast fatty liver is associated with an up regulation of markers of inflammation and oxidative stress. Strategies to minimise the risk of cows developing fatty liver *post-partum* are: reduce excessive deposition of fat during dry period, control BCS, enhance insulin sensitivity maintaining NEFA concentration under pathological range. Although BCS *pre-partum* provides a practical evaluation of the degree of body fatness degree, recent evidence has indicated that overfeeding energy can lead to a marked increase in visceral adipose tissue mass without appreciable differences in BCS or bodyweight, underscoring the importance of nutritional management to avoid diseases associated with over-conditioning (Overton and Waldron, 2004).

HYPOCALCAEMIA

Also known as milk fever, hypocalcaemia, is one of the most common bovine metabolic disorders consists in lowered level in calcium blood about time of calving, about 90% of milk fever occurs in the 24h after parturition. Physiological blood plasma Ca concentration has to be between 2.1 and 2.5 mmol/L, (Goff, 2008) under this limit is considered a subclinical stage of milk fever and under 1.4 mmol/L clinical sign of hypocalcaemia begin to manifest (Venjakob et al., 2019). A nutritional approach to managing milk fever involves monitoring specific elements in the diet. A more alkaline (higher blood pH) metabolic state which has been associated with an increased incidence of milk fever is due to a failure in the dietary cation anion difference (DCAD). Cation like sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) have a positive charge and promote a more alkaline blood pH, while anions like chloride (Cl), sulfur (S) and phosphorus (P) promote a more acid metabolic state reducing the risk of milk fever. The anion metabolic effect consists in a higher Ca mobilization by the cow's bone reserve, better prepare her the time when calcium will be lost in milk. At the onset of lactation, cow's requirements for calcium quadruple. The reason that there are various anionic products anionic salts based on the market is to reduce the incidence of milk fever, lowering blood pH in close-up dry cow nutrition. The DCAD is determined by the cation-anion status of the ingredients, also water high in cations or anions could affect the ion balance. Symptoms of milk fever like a reduced muscle tone and a reduced appetite can lead to lots of associated disease like ketosis, abomasum displacement, uterine prolapse, retention of placenta, lameness and metritis. (Overton and Waldron, 2004)

DOWNER COW SYNDROME

The downer cow syndrome is a problem which has a remarkable prevalence in dairy farms nowadays. The causes can be many, in post-partum period generally the most frequent problem involves metabolism, although the causes can be also related to the musculature and skeletal system by the fact that there is a ligaments relaxation at the time of parturition. Disorders that can cause Downer Cow Syndrome can be based on minerals deficiency, related to calcium, phosphorus, magnesium or potassium concurring in clinical/subclinical hypocalcaemia. A certain prevalence has been found in the association of this syndrome with mastitis, placental retention, or with problems related to hepatic metabolism (Overton and Waldron, 2004).

IMMUNOSOPPRESSION AT THE BASIS OF MASTITIS AND METRITIS

As we have already discussed, the transition period, although short, is when the majority of metabolic and infectious diseases occur during the dairy production cycle (Roche et al., 2013). Inflammation and dysregulated immune responses have been proposed as the missing link in the pathobiology of metabolic disorders in transition cows (Esposito et al., 2014). The peripartum itself represents a sensitive time in lactation period. In fact, the immune system is impaired during the transition period due to physical, hormonal and metabolic stresses associated with gestation, parturition and the onset of lactation. Numerous studies have reported decreases in immune-cell concentrations in blood reduced gene expression for immune components and impaired chemotactic and phagocytic capabilities about the time of parturition when compared with mid-late lactation. The state of NEBAL is often characterized by impaired neutrophil function including trafficking, phagocytosis and killing capacity. Lymphocyte numbers decrease about the time of parturition as a function of reduced proliferation. There is some *in vitro* evidence indicating that a marked elevation in blood NEFA and BHBA concentrations is one of the causative factors of immune suppression. Ketone body concentrations similar to those evident about the time of parturition also impaired the phagocytic and bactericidal capacity of neutrophils, effectively reducing udder defense mechanisms against mastitis pathogens. Other studies supports that that the event of parturition and associated metabolic changes contribute to the immune dysfunction, but that the lactation-induced NEBAL post-calving sustains the suppression of immune-cell function.

In addition to the sharp decrease in the number of circulating polymorphonuclear (PMN) neutrophils, the decrease in mammary T- lymphocytes from late pregnancy through calving impairs the cow's ability to fight pathogens during the early *post-partum* period. The impairment of PMN-neutrophil function during the transition period exacerbates susceptibility to mastitis and metritis.

In addition, other mechanisms are involved in the immunosuppression process, like the change in blood steroid concentrations that, at the time of parturition contributes to impaired PMN-neutrophil function because it decreases the expression of genes and membrane proteins associated with apoptosis, protein translation, normal respiratory metabolism and the adhesion receptor L-selectin. Thus, hormonal changes at the time of parturition affect PMN-neutrophil function at a molecular level. However, the periparturient hormonal changes are short-lived and are unlikely to explain all of the alterations in PMN-neutrophil function throughout the transition period. Lastly, the decrease in circulating blood glucose with the onset of lactation is another possible factor associated with the impaired immune function after calving (Roche et al., 2013).

It is clear that the establishment of microbial infections in the reproductive tract can have negative consequences for reproductive function of the *post-partum* female. Compromised systemic immune function is a cause of uterine diseases. Another important factor that needs to be consider in the pathogenesis of the reproductive tract is that the early *post-partum* female has also just completed a prolonged period when immune function in the uterus is suppressed because of the need to limit maternal immune responses against the allogeneic conceptus. Among the regulatory processes responsible for inhibition is an immunosuppressive role for progesterone as well as differentiation of regulatory immune cells that can inhibit inflammation and T cell responses. Some of the immunological adjustments to the presence of the conceptus also make the uterus more susceptible to

bacterial infection. Thus, for example, progesterone not only depresses skin graft rejection but also reduces uterine capacity to eliminate bacterial infections. The macrophages can inhibit inflammation and facilitate persistence of some microbial infections. The complex process of uterine involution begins immediately after calving and involves uterine contractions, physical shrinking, necrosis, sloughing of caruncular material, and regeneration of the endometrium (Roche et al., 2013).
In uterine defense mechanisms defense against bacterial contaminants is provided by uterine leukocytes and PMNs that are the cells phagocytizing and clearing bacteria, and these cells are recruited at this level by chemotactic factors such as IL-8.

In establishing disease like metritis and endometritis it is firstly important to consider what happens at the time of calving: the vulva and cervix, which function as physical barriers together with the vestibule and vagina during pregnancy, relax and dilate, thus permitting bacterial influx into the uterus. As such, intra- and *post-partum* bacterial contamination of the uterus is inevitable. Immediately *post-partum* therefore the endometrium is the first line of defense against bacterial invaders.

Puerperal metritis has been defined as an inflammation of the uterus resulting in systemic signs of sickness, including fever, red-brown watery foul-smelling enlarged uterus with a watery or purulent discharge, dullness, inappetence, elevated heart rate, and low production in the first 7 days after calving. Other than the immunosuppression due to the transition period, there may be other predisposing factors such as retained placentas, fetal maceration, or difficult calving.

As well as the uterus, also the mammary gland is susceptible to bacterial infections like the mastitis. In fact, from 2 weeks prior to calving, until about 2–3 weeks after calving, is the most critical period for the health of the mammary gland (Pyörälä, 2008).

OVARIAN CIST

A combination of signs and symptoms of hormonal disorder and ovarian dysfunction characterize the ovarian cist, described as an anovulatory ovarian structures with a cavity greater than 20 mm in diameter in the absence of a corpus luteum. Exist two different types of ovarian cysts: follicular and luteal. It is however evident that the etiopathogenesis of ovarian cysts in dairy cattle is a complex process which implies the alteration of various physiological processes (folliculogenesis, steroidogenesis, ovulation) and some factor like stress, herd management, nutritional status, body condition and metabolic disorder. It remains difficult to identify the exact mechanism that generates this disorder. Some risk factors for developing ovarian cysts include also puerperium disorders such as twinning, retained placenta, primary metritis, and ketonuria. In addition, a high milk yield from the puerperium period is a risk factor for developing ovarian cysts (Borş and Borş, 2020).

SUBACUTE RUMINAL ACIDOSIS

The subacute rumen acidosis (S.A.R.A.) is a cow's disease that can occur during *peripartum* period usually characterized by abnormal and intermittent drops in rumen pH. The risk of develop this ruminal pathology is likely to arise when a palatable and high-energy diet meets a ruminal microbiota not adapted to this type of substrate. This typical event could show up during transition period, when the diet change from the dry of to the close up, preparing the animals for the high energy request of lactation. A transition in diet too fast or not well managed can be a cause of S.A.R.A. When the animal ingests concentrated feed, the volatile fatty acids are produced faster than their absorption by the mucous epithelium of the rumen. An increase of short-chained fatty acids, may result in a transient nadir of ruminal pH below 5.5. Furthermore, high energy diet with a low fibrous content do not sufficiently stimulate rumination and salivation, thus preventing the animal from naturally restoring the normal pH of the rumen (6-6.4) (Kleen et al., 2003). S.A.R.A. is a condition characterized by ruminal pH <5.6 for an extended period and is correlated with various health problems in dairy cows, such as feed intake depression, reduced fiber digestion, milk fat depression, diarrhea, laminitis, liver abscesses, increased production of bacterial endotoxins, and inflammation (Nagata et al., 2018).

ASSOCIATION BETWEEN MINERAL VITAMIN NUTRITION AND METABOLIC DISORDERS.

Minerals and vitamins administered in the cow's diet are not just essential components necessary for meeting minimum requirements to avoid deficiencies. A good nutrition of minerals and vitamins in fact, allows for mammary gland development, growth of the developing calf, and supports immune function. Nutritional programs that deliver optimal amounts of minerals and vitamins can promote good health and productivity above all in the transition period (Krys et al., 2009).

Table 1. Metabolic problems and minerals associated.

Pathology	Affected by mineral(s):
Ketosis	Co
Milk Fever	Ca, P, Mg, Na, K, Cl, S
Retained Placenta	Ca, Se
Displaced abomasum (DA)	Mg, Ca

Mineral abbreviations: Co (cobalt), Ca (calcium), P (phosphorus), Mg (magnesium), Na (sodium), K (potassium), Cl (chloride), S (sulfur), Se (selenium).

KETOSIS AS AFFECTED BY COBALT

Elemental Co is used by bacteria to synthesise vitamin B12. The mineral affect on ketosis is due to the central role that cobalamin (Vit. B12) has in the metabolism of lipid by the liver. Vitamin B12 act as a co-factor in the transformation of methylmalonyl CoA to succinyl CoA, which is then used in the Krebs cycle within the liver for the synthesis of glucose from propionate. A cobalt and Vitamin B12 insufficient tissue supply, therefore results in an accumulation of ketones in the blood. For that reason, sufficient dietary supply of Co during transition period (Table 1) plays a central role controlling Ketosis and SCK (Weerathilake et al., 2019).

MINERALS BALANCE IN MILK FEVER

As shown in Table 1, the risk of milk fever depends on dietary levels of a few minerals: sodium, magnesium, phosphorus and potassium are at the base of the dietary cation anion difference (DCAD) (as calculated by $[Na^{1+} + K1^{1+}] - [Cl^{1-} + S^{2-}]$), while calcium, phosphorus, and magnesium are all directly involve in Ca homeostasis. As reported before higher cations plasma level increases the incidence of *post partum* milk fever, while higher level in anions prevent it. A correct DCAD during transition period as to be approximately –15 mEq/100 g DM as reported by (Lean et al., 2013). In Table 2 are reported the mineral requirement for the maintenance of a correct DCAD plasma level during dry and transition period compared to requirements of cows fed a salts anionic diet.

PLACENTAR RETENTION AS AFFECTED BY CALCIUM AND SELENIUM

Retention of placenta (ROP), defined as the failure to expel the foetal membranes or placenta within 24 h of calving by the cow itself is one of the most common disease affecting cows in the *post partum* period. A partial retention of placentomes has a higher incidence than a complete retention. Short gestation length and low birth weight are associated with the high risk of ROP, such as high BCS at calving and toxemia. Despite cows with negative energy balance during *pre-partum* are 80% more susceptible to ROP also micronutrient deficiency is strongly associated at this disease, involving vitamin A, E, D and few minerals: Se and Ca. Imbalances in calcium homeostasis associated with hypocalcaemia, decreases the muscle tone in the uterus making the animal prone to ROP, the expulsion of placental membrane depend in fact on large part by uterus contraction. The biological effect of selenium on the other hand is exerted through its incorporation into selenoproteins, which are involved in the activation, proliferation and differentiation of cells that drive innate and adaptive immune response. Se is also involved in antioxidant systems such as glutathione peroxidase that immunity cells use to counteract high levels of oxidative stress. For these reasons ROP can be hardly reduced by prevention of hypocalcaemia and maintaining an adequate Se status in dairy cows (Swain et al., 2013).

Symptoms of milk fever like a reduced muscle tone and a reduced appetite can lead to lots of associated disease, between them, the abomasum displacement. Clinical or subclinical hypocalcaemia after parturition may determinate sufficiently low calcium plasma level to alter the physiological muscular tone of the abomasum. As reported in (Rodríguez et al., 2017) a subclinical hypocalcaemia (Ca plasma level < 2,14) increase 4.3 time the risk of develop a displaced abomasum in cows. Magnesium instead, is a fundamental element in a calcium homeostasis.

Table 2. Nutritional macro elements requirements in dry and transition cows (NRC, 2001) .

Mineral	Far-off dry cow (% of DM)	Transition cow	Transition cow (with anions)
Ca	0.6	0.7	1.4
P	0.26	0.3	0.4
Mg	0.16	0.2	0.4
Na	0.1	0.1	<0.1
K	0.65	0.65	<1.5
Cl	0.2	0.2	0.8
S	0.2	0.2	0.4

Mineral abbreviations: Ca (calcium), P (phosphorus), Mg (magnesium), Na (sodium), K (potassium), Cl (chloride), S (sulfur). (NRC 2001)

Table 3. Nutritional micro elements need in dry and transition cows (NRC 2001).

Trace Mineral	Far-off dry cow (PPM)	Transition cow (PPM)
Co	0.11	0.11
Se	0.3	0.3
Zn	60	60
Cu	12	18

Mineral abbreviations: Co (cobalt), Se (selenium), Zn (zinc), and Cu (copper). (NRC 2001)

EFFECTS OF VITAMINS IN THE TRANSITION PERIOD

At the time of calving, as we have already widely discussed, there is a strong depression of the immune system, which associated with a metabolic imbalance prone animal during transition period to typical disease and metabolic disorders. The processes of calving and lactation are proinflammatory, and a release of inflammatory mediators from lipid mobilization, environmental stressors, subclinical disease increase free radical levels, perturbing the homeorhetic and homeostatic responses of the organism to face up the energy demand of this period. At the time of calving serum concentration of fat-soluble vitamins retinol (Vitamin A) and α-tocopherol (Vitamin E) decline because of the high colostrum output of these molecules. For that reason a vitamins supplementation is required to support an enhancing effect on immune-cell function, combined with the antioxidant activity of these molecules (Lean et al., 2013).

Table 4. Nutritional vitamin requirements in dry and transition cow (NRC 2001).

Vitamin	Far-off dry cow (% of DM) IU/day	Transition cow IU/d
A	100	100
D	30	30
E	1200	2000
Niacin	6-12 g	6-12 G

Vit. E maintains proper antioxidant status of animals and improves the ability to resist infections in periparturient cows resulting in optimal udder health. Low plasma concentration of α-tocopherol at parturition is considered as a significant risk factor for intra-mammary infection and mastitis during first week of lactation.

Vitamin A or retinol is a fat-soluble vitamin involved in multiple mechanisms including the maintenance of epithelial cells, vision, gene regulation and immune cell functioning, which increases lymphocyte function and enhances the phagocytosis capacity with particular reference to neutrophils. The effects of Vitamin A deficiency are given by fertility disorders, visual disorders and the increase in the incidence of metritis, mastitis and placental retention in post-partum, following a reduction in the efficiency of the immune system (Weiss, 1998).

Vitamin PP also called Niacin plays a very important role as a coenzyme in the metabolism of carbohydrates, lipids and proteins. In rations with a high lipid content, it is useful in counteracting the consequent drop in the protein percentage of milk; it is also effective in the prevention of ketosis thanks to its role in glucose synthesis.

Vitamin D or 1,25-dihydroxycholecaciferol is involved in the homeostasis of calcium and phosphorus (as explained in the chapter on calcium homeostasis) and has a secondary role in both innate and adaptive immune responses. Although cattle exposed to sunlight may obtain sufficient vitamin D, it is clear that housed cattle do not unless supplemented. It is important in the prevention of hypocalcemia. A deficiency of this vitamin occurs when the plasma concentration is below 5 ng / ml, and leads to decreases in both milk production and reproductive performance (Weiss, 1998).

NUTRITION

Over the past decade, interest in carbohydrates in the transition cow has increased. Especially, several studies have focused on its nutrition whose efforts promote a positive transition period for the animals. Indeed, the transition period is the most delicate phase for management of dairy cow feed. The animal in this period presents a fragile immune-endocrine-metabolic status so any food errors can compromise it promoting the development of pathologies, reducing milk production and fertility. Nutritional needs during the transition period quickly increased due to two main reasons: to meet the needs of the foetus and to produce colostrum and milk. It must be considered that, at this stage, there is an up to 30% reduction in food intake and, in addition, the ability of rumen mucosa to absorbs nutrient is not yet optimal. For this reason, it is important to adopt feed strategies aimed to satisfy the needs of the animals without compromising the ruminal and hepatic functions. It has been seen that promoting high ingestion both in dry and lactating period allows to improve the nutritional balance conditions of the animal acting in prevention against the onset of a number of diseases. The main nutritional intervention to be implemented is to provide balanced energy, lipid and protein supply (Bertoni, 2003).

GLUCIDES

As already mentioned, the use of nutrients by the foetus and the mammary gland places considerable nutrients demand on the cow during the last period before calving. However, it has been shown that providing adequate non-fibrous carbohydrates (NFC) as energy source may provide benefits during this delicate phase for the cow. NFCs are able to promote different areas, including the ability of NFC to stimulate ruminal papillae development (Dirksen et al., 1985). When highly fermentable carbohydrates are introduced into the diet, the production of volatile FA increases, reducing rumen pH. The microbial population, in response of this variation, undergoes a selective pressure that must be gradually addressed in order to maintain a correct fermentation. Many studies, including the one of Weimer et al. (2010) demonstrated that gradual adaptation to the post-calving diet is possible, especially if there is a high different in NFC content from the pre-calving ration. The supply of glucogenic precursors reduces the catabolism of stored labile proteins and reduces excessive lipid mobilization from fat reserves. Furthermore, a correct administration of carbohydrates leads to an increase in rumen propionate which stimulates insulin. Insulin, in its turn, has an effect on the storage of NEFAs in the adipose tissue and suppression of lipolysis thus preventing disease such as hepatic steatosis (Overton and Waldron, 2004). However, non-fibrous carbohydrates must be given in the correct amounts in the cow diet: a higher dosage cause dangerous drops in rumen pH resulting in acidosis. Indeed, acidosis is a very frequent disease in livestock where a proper diet is not administrated during the transition stage. Basically, it is important using quantities of feed not exceeding 40% of the ration.

LIPIDS

A high interest during the last years has been given to the effect of lipid supplementation in dairy cows in the *pre-partum* period to increase hepatic metabolism and animal welfare. Traditional recommendations are around 3/4% lipid supplementation taking care not to exceed 6% of DM as total lipid. Basically, there has been an interest in the use of long fatty acids (LCFA) to manipulate lipid metabolism at the time of calving and prevent the accumulation of TAGs. A positive effect was demonstrated in increased expression of the microsomal triglyceride transfer protein and Apoprotein B (ApoB) enzyme, both of which are essential for VLDL synthesis. Linoleic acid, combined with palmitic acid, produced higher rates of gluconeogenesis by reducing the production of TAG from propionate. Among the oils used for LCFA supplementation, linseed oil reported a positive trend leading to lower NEFA production and lower TAG accumulation. The potential effects of LCFAs could go beyond hepatic and adipose tissue metabolism: promising works on rodents have shown that very long chain PUFAs can reduce the production of inflammatory cytokines through effects on gene expression. Similar results are being obtained on hepatic metabolism in ruminants (Overton and Waldron, 2004).

PROTEIN

Especially during the transition period, there is specific needs of aminoacids which are used for the *neo*-synthesis of glucose as gluconeogenetic precursors. According to (NRC, 2001) the nitrogen needs of rumen microbial population are met when the protein concentration of the ration reaches 12% of dry substance. Rumen degradability should be around 68-70%, and soluble proteins should be around 30-35%. During the early and middle stage of the transition period, nitrogen concentrations are easily reached by using good quality forages. As calving approaches, due to the increase in needs and the reduction of appetite, it is assumed the need to administer a diet with higher protein concentration. Because of their as glucose precursors, is also important to prevent a reduction in the availability of aminoacids such as alanine and glutamine which normally provide the body with 40% and 60% of glucose obtained from this way. In the majority of situations, during the transition period it is appropriate to increase the protein content to 13,5-14,5% of the dry matter with a 35% by-pass in order to balance the supplies also counteracting the reduced ingestion that occurs in this phase. Methionine and lysine are used more frequently as they appear to limit the production of large quantities of milk (Possibile et al., 2003).

CHAPTER 2: Experimental Contributes

INTRODUCTION

The transition period is a phase in the dairy cow life's that is commonly defined as the period from 3 weeks before to 3 weeks after calving (Esposito et al., 2014). That period comes after the dry phase and prepare the animal to afford the lactation period. After calving in effect, the output of nutrients with colostrum and milk overcome the input by feed intake (Ceciliani et al., 2018), creating a negative energy balance (NEB) constraining the animal to a massive mobilization of body reserve including fat, protein, minerals and vitamins. The problems that can result from this lack in metabolism homeostasis include hypocalcaemia, the downer cow syndrome, hypomagnesemia, ketosis, udder edema, abomasum displacement, metritis and poor fertility (DeGaris and Lean, 2008).

Diets formulated for a specific animal group could sometimes not be able to satisfy all the individual nutrient requirements and this is a critical point above all in the transition period, whose proper management is recognized to affect the entire subsequent lactation.

Ensuring a correct energy and nutrient supplementation has recognized to be an important step for the conduction of this delicate phase. In fact, the higher disease incidence (shortly after parturition) corresponds with the time of greatest negative energy balance (NEB), the peak in NEFA (non esterified fatty acids) blood concentrations, and the greatest increase of milk yield (Cardoso et al., 2020). Various nutritional strategies have been tested and different tools have been developed to help breeders in preventing reproductive and metabolic disease which punctually occur in the fresh cows.

Some strategies in prevention of hypocalcaemia and ketosis consist in providing a fully acidified negative dietary cation-anion difference (DCAD) diet *pre-partum*, with appropriate dietary Ca concentrations (2.0% of DM), which like as a dietary supplementation of cows with rumen-protected methionine and rumen-protected Lys improved health and reproductive performance of dairy cows after parturition (Cardoso et al., 2020). The first method helping the animal maintaining a correct calcium homeostasis, the second one sustaining milks' protein synthesis.

Other authors to oppose the lower DMI of periparturient cows tested a molasses-based liquid feed in a high-straw dry cow diets that was demonstrated to be an effective strategy for increasing DMI, reducing feed sorting, and promoting more consistent intake in the peripartum period. Furthermore, molasses shown an improve rumen health, demonstrated by higher mean reticulorumen pH in the transition period, associated with a better microbial growth granted by a sugar supplementation, especially for fiber-digesting bacteria (Havekes et al., 2020).

Another strategy in prevention of ketosis, in opposition of fatty acid mobilisation that lead to an increase plasma level of BHBA an NEFA is the use of glycerol in the feeding of ruminants. As reported in (Kupczyński et al., 2020) glycerol can be administered as a source of energy in the ration and as a glucogenic precursor. The high energy value of glycerol provides the opportunity to use this raw material as a partial grain substitute in cattle feed rations. Glycerol is reported to increases plasma glucose and may reduce non-esterified fatty acids and β-hydroxybutyrate levels.

In a study achieved by Muller et al. (1977) a mineral-vitamin free choice supplementation was experimented in dairy cows. The final results concluded that ruminants do not consume sufficient amounts of free-choice minerals and vitamins to meet their requirements but the palatability of vitamins rather than the craving for minerals influences free-choice consumption. Similar data were collected also by Ishler (2001). Feeding minerals and vitamins in a blended complete feed is a more certain method of insuring a correct nutrients administration.

The use of free choice energetic mineral-vitamin supplements for dry and transition cows administered as *ad libitum* as experimented by Righi et al. (2016) showed an increase of

DMI and nutrients availability during dry and transition period, improving early lactation cows performances. Furthermore, better parameters were evaluated on milk production, health status and reproductive function resumption.

A strategy widely used to enhance reproductive performances consist in an administration in peripartum period of dietary fats rich in n-3 PUFA which are highly present in linseed oil. The latter acts improving dietary energy density, influencing follicular growth and ovulation; increasing the number, diameter, and the life span of the corpus luteum increasing concentrations of progesterone and early *post partum* ovarian cyclicity (Jahani-Moghadam et al., 2015).

The aims of the present work were to evaluate the use of a free choice energetic mineral-vitamin supplement as a tool for the nutritional management of dry and transition cows and to study its effect on early lactation performances.

MATHERIALS AND METHODS

PRODUCTS TESTED

The dietary protocols included free choice cafeteria style mineral vitamin supplements (FCC) formulated for dry and lactating cows whose composition are reported in Table 5. They are low moisture blocks including a crystallized blend of molasses, proteins, fats, vitamins and trace minerals specifically formulated for each life phase as declared by the producer (Crystalyx® Products GmbH, Münster, Germany). The blocks were administered *ad libitum* as solid licking feeds holded in a 23 Kg of plastic parallelepiped shape container and placed in the paddock. The products tested during the dry period were Trockensteher® (T) and Trockensteher LIN/MOS® (L), while the product tested during the lactating period was Vitalyx® (V). Three dietary protocols were tested: TV= T followed by V, LV= L followed by V and control (CON).

Table 5. Chemical composition of the product as reported in the label.

	Vitalix	Trockensther LIN/MOS	Trockensther
Ingredients			
	Molasses	Ruby molasses	Molasses
	Glycol propylen	Magnesium oxide	Magnesium oxide
	Monocalcium phosphate	Sodium chloride	Sodium chloride
	Sodium chloride	Magnesium phospahate	Magnesium phosphate
	Vegetable fatty acid	Linseed oil	Vegetable fatty acid
	Calcium carbonate	yeast	Magnesiumsulphate
	Magnesium phospahate	Magnesium sulfate	Monocalciumphosphate
		Monocalcium phosphate	
Chemical composition			
Crude protein (%)	4,00	7,00	4
Crude fiber (%)	0,05	0,30	0.01
Raw fat (%)	6,50	2,50	2,5
Raw hash (%)	31,00	35,00	36.00
Potassium (%)	4,00	-	2.40
Phosphorum (%)	2,00	1,30	1.30
Sodium (%)	3,00	4,00	4.00
Magnesium (%)	1,00	8,00	8.00
Calcium (%)	2,20	2,10	-
Sugar (%)	32,00	32,00	32.00
Humidity (%)	5,00	5,00	5
Copper sulfate pentahydrate (mg)	520	750	750
Copper glycinechelate-hydrate (mg)	80	-	-
Manganese oxide (mg)	800	1500	2000
Zinc chelate hydrate (mg)	900	-	1500
Zinc oxide (mg)	300	2500	2500
Iodine (mg)	60	100	100
Cobalt carbonate (mg)	12	10	10
Selenium (mg)	20	27	27
Selenium metionine (mg)	-	3	3
Vitamin A (IE)	30000	400000	400000
Vitamin D3 (IE)	20000	90000	90000
Vitamin E (mg)	150	3000	3000
Vitamin B1 (mg)	135	-	-
Niacin (mg)	1000	-	-
Saccharomices Cerevisiae (KBE)	150*10^9	-	-

EXPERIMENTAL DESIGN AND DIET

The experiment was conducted in a commercial dairy farm located in Northern Italy (Lat 45.0528, Long 10.9313). The herd included 180 lactating Italian Holstein cows divided into 4 homogeneous pens, each exchanging cattle with a separate dry group located beside it. The cows in each lactating group were milked by a specific robotic milking system. The trial was conducted from December 2020 to August 2021 and involved 27 Holstein cows. Three of these pens were employed for the trial. More in detail, the animals were enrolled at the beginning of their dry period and randomly allocated in three homogenous groups. All the animals were observed for a period of 80 days, (from the drying of, at -60 days to calving at 20 days in milk- DIM). A basal complete diet was fed to all the dry animals providing 75% of the mineral vitamin requirements (Table 6).

The dry groups were categorized as follows: “T” group, supplemented from -60 to -7 DIM with CRISTALIX Trockensteher® (parity: 1.4; average daily milk yield in the previous lactation: 23.0 Kg); the “L” group supplemented from -60 to -7 DIM with CRISTALIX Trockensteher Lin/Mos® (parity: 1.4, with an average daily milk yield in the previous lactation of 24.4 Kg;) and a “CON” group, receiving as a top dressing the mineral-vitamin supplement to cover the remaining 25% of their mineral-vitamin requirements (parity: 1.3, with an average daily milk yield in the previous lactation of 24.0 Kg).

From 7 days before parturition the dry cows were moved into the lactating groups, and the cows of the two dietary protocols (TV and LV) received a product formulated for the lactating cows (VITALYX®). These During this period, all the animals were fed the same total mixed ration (TMR) formulated for lactating cows, whose composition is reported in Table 6. Moreover, starting from parturition, each lactating cow received supplemental feedstuff in the robotic milking system adjusted based on milk production and DIM.

Table 6. Ingredients and chemical composition of the diets supplied to the cattle during the dry and lactating periods.

	Lactating	Dry
Ingredients, % DM		
Grass hay[1]	8.6	78.1
Alfalfa hay 1 and 2[2]	43.0	
Corn flakes, 75 % starch	26.7	7.1
Soybean meal, 50 % CP	3.9	13.8
Mineral vitamin 1 and 2[3]		1.0
Sugar	2.1	
Beet pulp pellet	2.7	
Feedstuff from robot[4]	12.8	
Chemical composition, %DM		
DM, % as fed	87.3	87.3
ASH		4.7
CP	14.8	9.3
EE	1.6	1.52
STARCH	22.4	1.8
aNDFom	38.4	1.9
ADFom	28.8	47.8
ADL	5.0	29.4

[1] grass hay, DM % as fed 87.9: as a % on DM, 10.6 CP, 1.6 EE, 11.1 ash, 53.6 aNDFom, 31.5 ADFom, 4.5 ADL.

[2] Alfalfa hay 1, DM % as fed 86.5% DM: as a % on DM, 15.6 CP, 1.4 EE, 8.8 ash, 46,8 aNDFom, 31.6 ADFom, 5.1 ADL. Alfalfa hay 2, DM % as fed 89.4: as a % on DM, 19.2 CP, 1.3 EE, 9.4 ash, 45.4 aNDFom, 34.6 ADFom, 6.6 ADL.

[3] mineral vitamin 1 %DM: 86 barley meal, 10.49 vitamin premix, 1.06 Acid buffer celtic Sea minerals, 0.82 dry yeast from Saccharomices cerevisae, 0.17 calcium, 0.17 sodium chloride, 0.16 monocalcium phosphate 0.16 magnesium oxide, 0.08 magnesium chloride; mineral vitamin 2 %DM: 57.56 calcium chloride, 20.26 sodium chloride, 11.06 magnesium oxide, 8.87 acid buffer celtic Sea minerals, 0.95 vitamin premix, 0.88 barley meal, 0.10 magnesium sulphate, 0.10 sodium sulphate, 0.10 ground shells.

[4] feedstuff compositions %DM: 23.18 whole soybean meal flakes, 22.8 barley meal, 12.87 soybean meal 46%CP, 9.9 mineral vitamin premix, 8.02 corn gluten meal, 7.78 hard wheat flour, 4.97 beetpulp pellet, 2.96 dehydrated alfalfa meal, 2.67 sodium bicarbonate, 1.54 dry yeast, 1.48 magnesium oxide, 0.56 sodium chloride, 0.55 sucrose, 0.53 Dicalcium Phosphate, 0.11 monocalcium phosphate, 0.05 potassium carbonate.

The amount of TMR delivered was recorded per group every three days, and the amount of orts was weighted at the same interval for the dry cows and the following day for the lactating cows.

Feed intake was calculated as a difference between the amount delivered and the orts weighted divided by the number of cows in the pen and number of days after distribution.

SAMPLING

Every fifteen days a sample of each dietary ingredients as well as a sample of the diet fed to both dry and lactating groups were collected.

The cows were sampled at -60, -7, 0, +6 and +20 DIM. At these timepoints, with exception for + 6 DIM, the body condition score (BCS -) and fecal score (FS -) were evaluated.

Body condition of each cow was scored using both visual and tactile evaluation, using a scoring system from 1 to 5 with 0.25 increments as described by (Edmonson et al. 1989), where 1 is thin (emaciated condition) and 5 is fat. The system is basically based on a palpation of the backbone, the lumbar processes and the tailhead region evaluating the sharpness, the fat and the muscle covering the bones. Visual approach completes the overview focusing mainly on transverse and spinous processes, tuber coxae and overhanging shelf of the rumen. A ΔBCS (final BCS – first BCS) was also calculated in three different intervals (from -60 to 0, from 0 to 20 and from -60 to 20 DIM).

The fecal score was evaluated directly from each individual assigning a point level according to Kononoff et al. (2002)and fecal samples were collected in containers for further analysis. At score 2 correspond watery feces, measuring less than 3 cm in height and forming not form a distinct “pile” when they fall to the ground splashing, the cause of this could be an insufficient amount of fiber supplied. With 3, that’s the optimal score we have a height between 3 and 5 cm, with a depression in the center and several concentric circle around. At score 4 (typical of dry and older heifers) feces are thicker and higher than 5 cm, that's probably due to the low quality of the fodder and a lack of protein in the ratio.

Urine samples were taken after perineal stimulation avoiding to collect the first aliquots. The pH of urine and feces was measured using a pH-meter.

Blood samples were collected from the coccygeal vein using 2 vacuum tubes and changing needle each cow. Blood samples were centrifugated and the plasma frozen at -20 ready for later analyses. At 6 DIM only a blood sample was collected from each cow for the metabolic profile evaluation.

Colostrum samples of the first milking was collected by the farmer in a sterile bottle and its IgG quality was evaluated using a refractometer (automatic thermal compensator; Tecnolatte S.r.l).

Milk yield of the first two milking, as well as the daily milk yield, daily minutes of the rumination time and, the time spent eating were recorded by the Lely software for further evaluations.

FEED ANALYSIS

Prior to the analyses, feed and diet samples were dried at 55 °C to constant weight, then ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass 1 mm screen as described in Simoni et al. (2020). The dry matter (DM) content was determined at 103 °C overnight. Ash content was measured gravimetrically by ignition at 550 °C (Simoni et al., 2020). Crude protein (CP) content was calculated as a percentage multiplying the N content *6.25. The latter chemical compound was determined by the combustion digestion of the sample at 900 °C in excess of oxygen by Dumatherm® (Gerhardt GmbH & Co, Königswinter, Germany) as described by (Mihaljev et al., 2015). Ether extract (EE) was determined using the Soxhlet extraction system and starch content was determined by polarimetric method (European

Commission, 2009). Fiber fractions (aNDFom, neutral detergent fiber assayed with a heat stable amylase expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; lignin (sa), lignin determined by solubilization of cellulose with sulphuric acid) were analyzed according to Van Soest et al., (1991) using a semi-automated system for the boiling and filtering phase (FI-WE Raw Fibre Extractor, VELP Scientifica, Usmate Velate, Italy), and expressed as exclusive of residual ash. No sodium sulphite was used for aNDFom determination.

METABOLIC PROFILE

Under veterinarian control, blood samples were collected After the in Lithium-heparin (LH) tubes at -60, -7, 0, +6 and +20 DIM, centrifuged at 3000 rpm for 10 min and the obtained plasma was analyzed to obtain the metabolic profile including total protein (Tp), urea (Ur), glucose (Glu), cholesterol (Chol), triacylglycerol (Trig) and minerals (Ca, Mg and P). To monitor the energy metabolism of animals were also determined NEFA and BHBA.

Analysis was conducted using an ILab 650 automatic multiparametric analyser for clinical biochemistry (Instrumentation Laboratory Company, Lexington, MA, USA) with the related reagents. All the analysis are conducted at 37 °C.

CALCULATION

Time spent ruminating related to the milk yield was calculated dividing the minutes spent ruminating by the kg of milk yield of each day for each cow.

STATISTICAL ANALYSYS

Statistical analysis was performed using the SPSS for Windows software package (version 26.0; SPSS Inc., Chicago, IL). Products intake has been analysed as a comparison among the two treated groups, while *pre-partum* and post-partum TMR intake were compared among groups through the univariate procedure of the generalized linear model (GLM) applied on the average daily consumption measured every three days in each group from January to August 2021. Group protocol was used as a fixed effect, and the comparison among groups was assessed by LSD post-hoc test.

Individual time spent feeding, individual time spent ruminating, milk yield time spent ruminating per kg of milk were analyzed per groups as a repeated measure, using the group as a fixed effect, differences among groups were evaluated using the Bonferroni post-hoc test. The interaction between group and interval was also tested.

The BCS, ΔBCS, fecal score, urine pH and parameters of the metabolic profile were analysed through the multivariate procedure of the GLM. The group protocol was used as a fixed effect and Bonferroni was applied as a post-hoc test.

Colostrum yield in the 1st and 2nd milking and BRIX degrees in the 1st milking were analysed with the MIXED model. Group was used as a fixed effect, parity of the dam, cow and days of dry period were random effects. The LSD post-hoc test was applied to compare the groups.

Statistical difference was set at $P < 0.05$, while value between 0.1 and 0.05 were considered as a trend.

RESULTS AND DISCUSSION

The intake of the products and of the TMR fed to the dry and lactating cows are reported in Table 7. During the far-off period a lower consumption was observed in TV protocol compared to the LV protocol (0.201 vs. 0.322 Kg as fed respectively, $P \leq 0.001$) while any difference was observed on the subsequent Vitalyx consumption during the lactating period (0.180 vs. 0.167 Kg as fed respectively, $P = 0.282$) even if the consumption in LV protocol tended to remain numerically higher.

Table 7. Free choice caffetteria style intake of the treated groups during dry and lactating period, and Total mixed ratio (TMR) intake as affected by the protocol Free Choice Cafeteria Trockensther followed by Vitalyx (TV), Free Choice Cafeteria Trockensther LIN/MOS followed by Vitalyx (LV) compared to a control group (CON).

	Dietary protocols			sem	P-value
	TV	LV	CON		
Intake of product supplied to dry groups[1], Kg/cow/d	0.201a	0.322b	-	0.01	≤0.001
Intake of product supplied to the lactating period[2], Kg/cow/d	0.167	0.180	-	0.006	0.282
Intake of TMR DIM -60 to -7, kg/cow/d	12.79a	14.46b	14.41b	0.221	0.002
Intake of TMR DIM -7 to +20, Kg/cow/d	24.10b	22.52a	23.22a	0.165	≤0.001

[1]The products tested during the dry periods were: Group 1= Trockehensteher®, Group 2 =Trockehenster LIN/MOS®

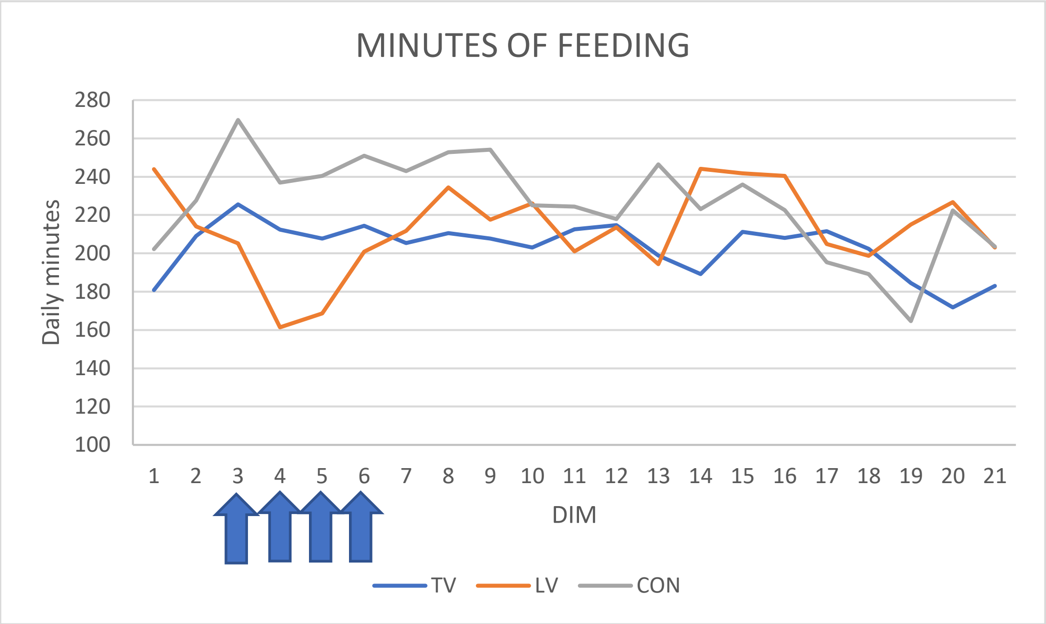
[2]The product tested during the lactating period was Vitalyx®

The TMR intake differed between groups, as reported in Table 7. In fact, during the dry period there's a higher consumption of TMR in the LV and CON protocols compared to the TV protocol (14.46 and 14.41 vs. 12.79 Kg as fed respectively, P = 0.002), while there is an opposite trend in the subsequent period (-7 to +20) when the TMR consumption become higher in the TV protocol (22.52 and 23.22 vs. 24.10 Kg as fed respectively, P < 0.001). Contrariwise, in a similar experiment based on a solid free choice mineral vitamin supplemented diet, no significant differences between treated and control groups were observed in hay intake during the whole dry period periods (Righi et al., 2016). However, the latter author showed a significantly higher average mixed hay intake in the treated group during the first 3 days of lactation (colostral phase). Similarly, in the present study, an increased TMR intake in the TV protocol compared to the CON protocol was found in the first 20 DIM. The lower TMR consumption in the TV protocols could be due to a decreased palatability of the Trockensther product tested that could have influenced the product intake such as the TMR intake of animals. This hypothesis can be supported by the fact that cows supplied with Trockensther LIN/MOS during the far off consumed more product (0.322 vs. 0.201 respectively P ≤ 0.001). Moreover, it should be highlighted that this latter group consume also a higher quantity of TMR (14.46 vs. 12.79 respectively P = 0.002). Additionally, a higher variability in the product consumption itself have been noticed and this could have affected the animals' intake during the whole observation period increasing the intake variability.

In the Figure 2 the time spent eating by the cows of each group during the first 20 DIM is represented. The feeding time (recorded by radio collars placed on each animal) was not affected by the treatments. Nevertheless, from day 3 to day 6 as shown in the figure, cows of the control group spent more time feeding compared to the treated cows. Despite the absence of significant difference, it should be highlighted that cows in the CON group spent more minute daily eating compared to the LV group (240 vs 168 min respectively) in the initial weeks of lactation. In the following period during lactation, the lines tend to overlap indicating that the feeding time became similar between groups. Furthermore, even if the line of CON and both treated protocols tend to overlap it should be remarked that the TV

TMR intake was higher compared to the LV and CON groups, indicating that the TV group cows were eating at a higher rate. Additionally, the LV cows spent less time eating giving a similar intake to the CON group suggesting a faster feeding induced by the treatment. The acceleration effect was demonstrated in a similar study on the supplementation with liquid molasses (Havekes et al., 2020). The latter experiment highlighted that treated cows ate faster due to a reduced feed sorting and tended to have also more meals per day. A similar effect is reported in (Boudon et al., 2007), where a group treated with rumen glucose infusion had more meals per days and spent less time feeding compared to a control group.

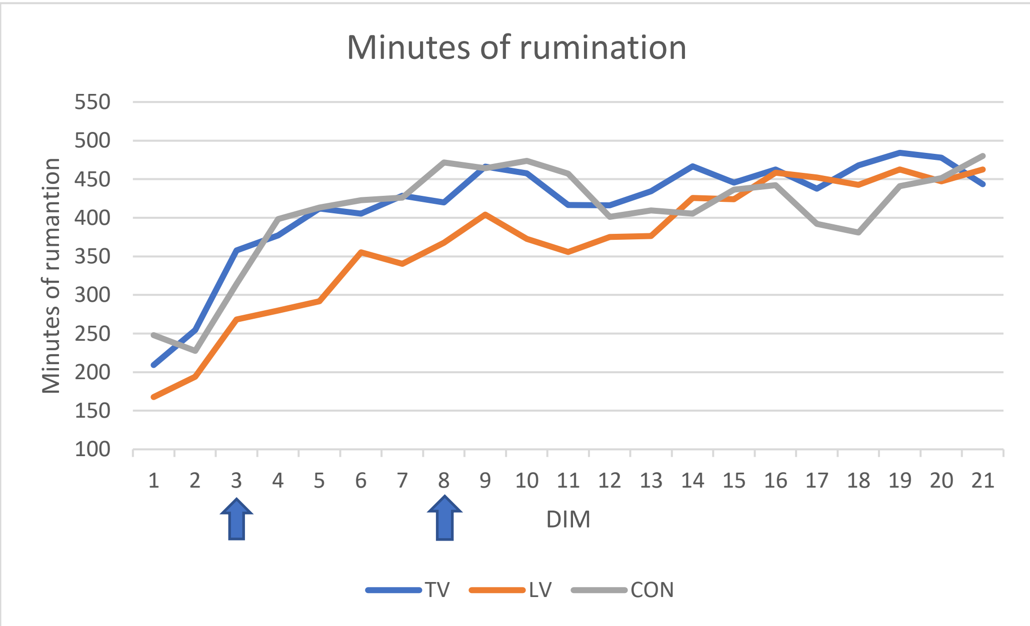
Figure 2. Daily minutes of feeding during the first 21 days in milk (DIM) as affected by the treatment Free Choice Cafeteria Trockensther followed by Vitalyx (TV), Free Choice Cafeteria Trockensther LIN/MOS followed by Vitalyx (LV) compared to a control group (CON).



In Figure 3, at 5 and 8 DIM the time of rumination had a lower trend in the LV protocol than CON and TV groups, showing a tendency 5 DIM (413.33 vs 291.88 min respectively, P = 0.05) and 8 DIM (471.67 vs. 367.50 min respectively, P = 0.08). As found by Havekes et al.

(2020) testing the effects of molasses-based liquid supplementation, cows fed TMR with molasses showed a low rumination rate per kg of DMI compared to the control group. In the present trial, the rumination rate per Kg of DM in the CON group was higher compared to both treated groups. A booster effect of liquid sugars on the microbial growth and on the fermentative activity was shown, as reported by Ciriaco et al. (2015). The author tested a mixture of molasses and crude glycerol 50:50 on beef heifers showing an increased fiber digestion due to a raised rumen activity which led to an increased ADG (average daily gain). The less rumination time spent by treated animals could be in part due to a higher digestive effect of the microbial fermentative activity on the long fiber, so the animal will have less material to ruminate.

Figure 3. Daily minutes of rumination of the first 21 days in milk (DIM) as affected by the treatment Free Choice Cafeteria Trockensther followed by Vitalyx (TV), Free Choice Cafeteria Trockensther LIN/MOS followed by Vitalyx (LV) compared to a control group (CON).



In Table 8 are reported the differences of BCS in the three different dietary protocols, at four different time points. Starting from a similar BCS among the three evaluated groups, the animals in the CON group reached a higher BCS at the parturition compared to the TV group (3.72 vs 3.22; P=0,036). The BCS of CON animals exceeds 0.5 the BCS of TV appearing at the same time numerically higher than the LV group (3.38). Nowadays, in the known herd management practices, it is recommended in high productivity dairy cows to have a moderate BCS (≥3.25 and <3.5) at the beginning of the peripartum period, consequently resulting in lower mobilization of body reserves (Wang et al., 2019).

Table 8. Body condition score (BCS based on a scale 1-5 as described by (Edmonson et al., 1989) at different time points (-60, -7, 0 and +20 Days in milk -DIM) as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

	Dietary protocols			SEM	P
DIM	TV	LV	CON		
-60	3.16	3.47	3.56	0.106	0.276
-7	3.44	3.69	3.64	0.075	0.379
0	3.22a	3.38ab	3.72b	0.088	0.036
20	3.09	3.00	3.05	0.090	0.933

The 3 different dietary protocol groups (TV, LV and CON) obtained a similar BCS 20 days after parturition, (3.09 vs. 3.00 vs. 3.05 respectively, P = 0.933). However, three different ΔBCS from parturition to 20 DIM were found as shown in Table 9 respectively 0.25 vs. 0.39 vs. 0.67; (P = 0.31). The higher variation in the CON BCS, lead to a higher probability to experience delay in the ovulation of the CON group cows due to higher reserve mobilization that can also increase the chance of developing ketosis. In fact, as reported in literature (Morales Piñeyrúa, Fariña, and Mendoza 2018) cows with a higher BCS loss (ΔBCS > 0.75 point) showed a higher probability of having a longer interval of first ovulation. The ovulation delay is mainly due to a higher metabolic imbalance due to a negative energy balance, that depending on its severity, can result in an increased incidence of metabolic diseases and infertility. In fact, it is estimated that up to 75% of disease incidence and economic losses in the dairy industry occur during this transition period (Morales Piñeyrúa et al., 2018).

Table 9. Body condition score (BCS) variation in three different intervals as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

	Dietary protocols			SEM	P
DIM	TV	LV	CON		
-60 to 0	-0.06	0.06	-0.17	0.096	0.655
0 to +20	0.25	0.39	0.67	0.112	0.313
-60 to +20	0.19	0.44	0.50	0.145	0.671

In Table 10 are presented the different fecal score with a difference at 20 DIM, when the feces of TV protocol are more fluid than the CON group (2.53 vs 3.36 point respectively, P = 0.023). The lower score of the treated groups could be due to a higher TMR intake of the TV protocol that could lead to a high dry matter (DM) content in feces that may have an osmotic effect on the gastrointestinal apparatus. A higher DM content in feces was found by Boudon et al. (2007) in an experiment where was compared to a control group an experimental group treated with ruminal glucose infusion.

Table 10. Fecal score evaluated at different time points as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

DIM	Dietary protocols			sem	P-value
	TV	LV	CON		
-60	3.34	3.41	3.56	0.075	0.545
-7	3.59	3.41	3.61	0.111	0.725
0	2.84	3.03	2.78	0.119	0.704
20	2.53a	2.68ab	3.36b	0.129	0.023
OVERALL	3.26	3.28	3.32	0.108	0.499

In Table 11 is presented the urinary, which shows no significant differences among the groups. This suggested that the product tested do not affect this parameter. The values were physiologically normal (between 8 and 8.5) as reported in literature (Constable et al., 2019).

Table 11. Urine pH measured at different time points as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

DIM	Dietary protocols			sem	P-value
	TV	LV	CON		
-60	8.17	8.14	8.17	0.061	0.985
-7	8.29	8.34	8.27	0.044	0.861
0	8.44	8.22	8.02	0.074	0.175
20	8.41	8.30	8.27	0.047	0.498
OVERALL	8.33	8.25	8.18	0.056	0.630

The colostrum quality could be evaluated by brix refractometry. As reported in literature (Gamsjäger et al., 2020) samples with Brix percentages <22° were between 11.2 and 16.8 times more likely to contain IgG <100 g/L than they were to contain IgG ≥100 g/L, and samples with Brix percentages of 22° to 23.9° were between 3.8 and 6.7 more likely to contain IgG <100 g/L than they were to contain IgG ≥100 g/L. Brix percentages of <24° therefore were optimal to detect colostrum with IgG concentrations <100 g/L for all laboratories. Brix percentages of ≥30° were optimal to detect colostrum IgG concentrations ≥150 g/L for all laboratories, because those samples were between 3.3 and 5.3 times more likely to contain IgG ≥150 g/L than they were to contain IgG <150 g/L. In our case even if there is not a significative difference between each dietary protocols, colostrum of LV and CON are average higher than TV in brix values. Was on average lower the colostrum quantity at first yield in the TV protocol (3.73 vs. 4.50 and 4.40) but no statistical evidence was found.

Table 12. Colostrum yield at first and second milking; and brix calculated only at the first time by refractometer as affected by the Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

	Dietary protocols			sem	P-value
	TV	LV	CON		
1st colostrum yield (Kg)	3.73	4.50	4.40	0.21	0.306
2nd colostrum yield (Kg)	5.28	5.38	5.01	0.32	0.889
Brix of the 1st colostrum (°)	21.34	24.77	23.76	0.76	0.120

Supplementing lactating cow with a mineral vitamin free choice had a depressive effect on milk yield from day 5 to day 10 with a tendency at day 6. As shown in Table 13 TV and LV protocols had a lower milk yield per day than CON group. Remind the higher BCS of the CON group’s cows at parturition, the higher milk production in early lactation could be associated to a higher fat mobilisation and a higher energy availability of these animals. At 20 DIM the BCS of the three different dietary protocols is similar and thus less effect is shown in the milk yield. On opposite in literature as reported by Righi et al. (2016) in a similar experiment, found an average milk yield and a daily milk production tendentially higher in the treated group compared to the control one. In (Havekes et al. 2020) where was experimented a supplemented administration of liquid molasses in dairy cows' diet, no difference was found between treated and control cows in the first four days of lactating period. To better understand the products effect it would be necessary to analyse the data of medium and late lactation.

Table 13. Milk yield (Kg) of the first 21 days in milk (DIM) as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

DIM	Dietary protocols			sem	P-value
	TV	LV	CON		
1	5.37	7.10	5.33	0.466	0.357
2	17.01	22.01	17.50	1.241	0.232
3	23.72	26.95	26.61	1.466	0.488
4	28.14	28.25	32.40	1.426	0.135
5	31.44ab	28.81a	36.70b	1.462	0.041
6	32.59	31.16	38.25	1.668	0.163
7	35.20a	34.74a	42.47b	1.547	0.029
8	35.90a	43.61ab	43.61b	1.744	0.016
9	36.56a	36.32ab	44.66b	1.788	0.020
10	38.35a	37.42a	47.89b	2.090	0.005
11	40.32	36.81	45.05	1.481	0.067
12	39.70	37.84	47.09	2.041	0.057
13	42.25	38.09	46.08	1.542	0.136
14	41.58	38.48	46.72	1.625	0.138
15	43.17	40.43	46.50	1.436	0.321
16	43.92	42.05	47.06	1.451	0.472
17	43.99	42.63	47.87	1.468	0.361
18	45.54	43.92	47.48	1.420	0.666
19	47.28	43.27	48.25	1.355	0.437
20	46.69	40.33	50.51	1.632	0.079
21	45.38	45.83	50.36	1.364	0.334
OVERALL	36.39	35.15	40.88	0.552	0.092

In Table 14 are shown the minutes of rumination per kg of milk yield. No differences were found with the exception of day 7 in which the LV group had fewer minutes of rumination than the TV group (9.07 vs 12.31 min per kg respectively, P = 0.021). Although there were no differences between the three groups, it should be highlighted that the control group from the 12 DIM spent less time ruminating per kg of milk compared to LV and TV protocols, indicating a greater probability of incurring ruminal diseases.

Table 14. Daily minutes of rumination per kg of milk yield of the first 21 days in milk (DIM) as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

DIM	Dietary protocols			sem	P-value
	TV	LV	CON		
1	45.35	37.90	50.28	5.254	0.730
2	15.81	12.64	13.25	1.594	0.681
3	15.10	10.75	12.87	1.628	0.381
4	13.52	9.46	12.29	1.526	0.258
5	13.42	9.43	11.28	1.144	0.141
6	12.90	12.11	11.56	0.861	0.769
7	12.31b	9.07a	10.04ab	0.891	0.021
8	11.83	9.67	10.86	1.017	0.324
9	12.97	11.03	10.39	1.577	0.069
10	12.13	9.86	9.90	0.962	0.196
11	10.45	9.21	10.22	0.534	0.729
12	10.61	9.79	8.55	2.189	0.136
13	10.46	9.03	8.74	0.683	0.465
14	11.49	11.11	8.53	0.664	0.089
15	10.42	10.38	9.08	0.542	0.559
16	10.70	11.05	9.12	0.580	0.333
17	10.07	11.25	8.00	0.554	0.071
18	10.31a	11.06a	7.76b	0.538	0.043
19	10.19	11.11	9.07	0.405	0.176
20	10.21	11.90	8.82	0.483	0.071
21	9.88	10.83	9.30	0.496	0.606
OVERALL	13.55	12.03	12.22	1.149	0.326

This trend is emphasized at day 18 when the control group has significantly less rumination per kg of milk than the other two groups LV and TV (7.76 vs 11.06 and 10.31 min per kg respectively, P = 0.043).

In Table 15 are reported the plasma concentrations of total proteins, urea, glucose, AST-GOT and GGT at different time points. The hepatic parameters (AST-GOT and GGT) were not affected by the treatment and resulted physiologically normal in the three dietary protocols at every interval (Brasca et al., 2012). At 6 DIM total proteins and glucose concentration were higher in the CON and LV group than in the TV group, (63.96 and 72.16 vs. 62.05 g/L respectively for total proteins, P = 0.020; and 3.39 and 3.76 vs. 2.98 mmol/L respectively for glucose, P = 0.020). The higher level of glucose at 6 DIM in the LV group could be due to an enhanced antilipolytic effect of insulin that Pires et al. (2008) demonstrated to have the linseed oil. This molecule is a source rich in n-3 C18:3 PUFA showing an antilipolytic effect and a depression of fat mobilisation, ensuring higher plasma glucose concentrations as reported in Jahani-Moghadam et al. (2015). Urea's plasma level on the contrary was significantly higher in the TV and LV group than CON at 6 DIM (4.20 and 3.80 vs. 2.91 mmol/L respectively). As reported by Tamminga, (2006) an excess of lipogenic nutrients may result in higher levels of non-esterified fatty acids (NEFA) in the blood and may accumulate in the liver. As a result, liver function may be impaired and the rate of conversion of ammonia into urea may be reduced.

Total proteins at 20 DIM where higher in LV group compared to TV (80.85 vs. 67.72 g/L respectively, P = 0.041).

Table 15. Metabolic profiles as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

Item	DIM	Dietary protocols			sem	P-vaue
		TV	LV	CON		
Total proteins, g/L	-60	73.85	79.75	76.09	1.031	0.102
	-7	67.69	71.61	68.77	1.293	0.572
	0	65.69	70.04	66.26	1.355	0.384
	6	62.05a	72.16b	63.96ab	1.570	0.020
	20	67.72a	80.85b	73.17ab	1.800	0.041
Urea, mmol/L	-60	5.3	5.8	5.77	0.204	0.590
	-7	5.03	5	5.93	0.297	0.169
	0	4.93	3.84	4.67	0.174	0.344
	6	4.20b	3.80ab	2.91a	0.216	0.039
	20	4.21	4.38	3.88	0.251	0.814
Glucose, mmol/L	-60	4.16	4.28	4.24	0.051	0.724
	-7	4.14	4.04	3.83	0.200	0.258
	0	3.75	4.58	3.93	0.106	0.198
	6	2.98a	3.76b	3.39ab	0.061	0.020
	20	3.32	3.69	3.43	0.067	0.140
AST-GOT, IU/L	\	115.87	87.13	79.83	6.942	0.150
	-7	90.43	77	71.83	21.254	0.177
	0	125.17	110.38	143	5.666	0.897
	6	91.63	94.38	102	3.318	0.795
	20	85.93	81.5	94.08	3.985	0.467
GGT, IU/L	-60	26.5	24.3	25.1	1.618	0.840
	-7	22.5	21.0	16.8	1.210	0.205
	0	24.7	20.4	18.9	4.206	0.330
	6	22.0	18.4	17.0	1.340	0.409
	20	28.0	22.0	26.9	2.568	0.704

Another important parameter is the plasma mineral content (reported in Table 16). The statistical analysis found a higher phosphorus concentration in plasma of LV compared to CON at two timepoints: parturition and at 6 DIM. No differences were found in the plasma level of the others two elements. It has to be considered that the CON group received the mineral supplementation in the feed bunk to satisfy cows requirements as reported in (NRC 2001) while the mineral supplementation of the two treated groups, LV and TV, was totally guaranteed by the free choice supplies. Based on results we can assume that with the administration of a free choice cafeteria style mineral vitamin supplements, cows are able to assume the correct quantity of these elements based on their requirements.

Table 16. Plasma mineral content as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

Item	DIM	Dietary protocols			sem	P-value
		TV	LV	CON		
Ca, mmol/l	-60	2.44	2.46	2.56	0.023	0.246
	-7	2.35	2.4	2.38	0.066	0.732
	0	2.02	2.3	2.16	0.046	0.367
	6	2.34	2.31	2.18	0.019	0.527
	20	2.47	2.43	2.35	0.037	0.631
P, mmol/L	-60	1.7	2.08	1.93	0.098	0.452
	-7	1.7	1.83	1.87	0.099	0.686
	0	1.27ab	1.63b	0.87a	0.088	0.006
	6	1.03ab	1.46b	0.74a	0.066	0.002
	20	1.74	1.53	1.16	0.095	0.172
Mg, mmol/L	-60	0.933	0.92	0.945	0.015	0.844
	-7	0.962	0.9	0.859	0.033	0.284
	0	0.998	0.815	0.891	0.024	0.133
	6	0.882	0.773	0.764	0.021	0.286
	20	0.939	0.954	0.877	0.030	0.450

NEFA and BHBA plasma level are evaluated to confirm possible clinical or subclinical ketosis: cows are defined as hyperketonemic when the concentration of BHBA in plasma exceeds the threshold of 1,200 to 1,400 μmol/L (van Knegsel et al., 2010) and to ketosis are correlated uterine infections and placental retention due to a depression of the immunity system caused by high levels of ketonic bodies (Steeneveld et al., 2020). In the present study, as shown in Table 17, the treatments decreased the BHBA content at -7 (0,40 and 0,41 vs 0,61 mmol/L respectively for TV, LV and CON; P = 0.012).

Table 17. Lipid metabolism as affected by the treatment Free Choice Cafeteria Trockensther® followed by Vitalyx® (TV), Free Choice Cafeteria Trockensther LIN/MOS® followed by Vitalyx® (LV) compared to a control group (CON).

Item	DIM	Dietary protocols			sem	P-value
		TV	LV	CON		
Cholesterol. mmol/L	-60	4.80	3.56	3.84	0.210	0.108
	-7	2.20	1.95	1.86	0.084	0.271
	0	1.69	1.74	1.61	0.114	0.874
	6	1.95	2.07	1.69	0.067	0.497
	20	3.58	3.37	2.88	0.170	0.394
Tryglycerides. mmol/L	-60	0.18	0.19	0.17	0.010	0.150
	-7	0.23	0.23	0.22	0.006	0.177
	0	0.12	0.12	0.13	0.005	0.897
	6	0.11	0.11	0.11	0.012	0.795
	20	0.11	0.12	0.11	0.004	0.467
NEFA. mmol/L	-60	0.40	0.24	0.23	0.053	0.564
	-7	0.32a	0.42ab	0.70b	0.076	0.031
	0	0.68	0.55	0.84	0.066	0.398
	6	0.54	0.54	0.54	0.059	1.000
BHBA. mmol/L	-60	0.46	0.46	0.50	0.031	0.890
	-7	0.40a	0.41a	0.61b	0.039	0.012
	0	0.71	0.58	0.75	0.046	0.225
	6	0.80	0.58	0.61	0.030	0.177
	20	0.58	0.52	0.52	0.039	0.911

Also, plasma concentration of NEFA at -7 DIM was significantly lower too in the two treated groups (0,32 and 0,42 vs. 0,70 mmol/L respectively; P = 0.031). The upper limits for NEFA reported by Brasca et al. (2012) are 0.68 and 0.82 mmol/L for dry and lactating cows respectively. Even if the threshold for BHBA was not reached, the NEFA content exceed the upper limit in the CON group, indicating a possible higher lipid mobilization with an increased risk of subclinical ketosis. As reported by Gerloff (2000) increased NEFA concentration in the peripartum period is observed in both hepatic lipidosis and ketosis. One possible explanation is that the accumulation of hepatic fat can lead to a decrease hepatic function including gluconeogenesis. As reported in literature (Kupczyński et al., 2020) propylenic glycol and others glucogenic precursor that are present in the *ad libitum* product administered can sustain the glucogenic metabolism against a high lipid mobilisation that could lead to ketosis.

CONCLUSIONS

The *ad libitum* supplementation with a mineral-vitamin blocks has proved to be a good alternative to help the metabolism of dairy cows in the critical transition period.

Particularly, an increased TMR intake in the post-partum period and a better BCS at parturition were found where the TV protocol was applied. Even if was recorded a minor feed intake during the dry period and a lower fecal score at 20 DIM, sign of a less efficient digestion, the milk yield at 20 is comparable to the CON group.

The LV protocol showed more similar parameters regarding herds' BCS and feed intake. However, even if a depression in the rumination activity was recorded in the first days after parturition compared to the CON and TV groups has to be highlighted the increased ruminal activity recorded per kg of milk in both the treated groups compared to the CON in the subsequent days, sign of a change in the behaviour.

Both the dry period supplements Trocknsteher® and Trockensteher LIN/MOS® increased the energy balance reducing the NEFA mobilization in the *peripartum*, helping the animals at the beginning of lactation when cow's metabolism dramatically changes, with an increase in tissue energy requirements often associated with a declining dry matter intake.

Plasma mineral concentration did not reveal any particular differences between the treated groups and the control group, demonstrating a good efficacy of the product supplying the correct mineral amounts. All these factors, associated with an extra vitamin supplement that only the treated cows received, could then have results in an improvement on the reproductive activity which was not evaluated in the present study.

The daily milk production in the first days was higher in the CON group but for a more correct analysis it is necessary to evaluate the whole lactating curve in the different groups and perform chemical-physical analyses of the milk to establish the real effect of these treatments on productivity.

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