Ruminant digestive physiology as research subject at the Instituto de Ciencia Animal for fifty years

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The Instituto de Ciencia Animal was created in 1965, with the objective of contributing to the development of Cuban cattle rearing under scientific and technical basis and different species of ruminants and non-ruminants were introduced in Cuba and constituted the subject for many researches. The objective of this paper is to report the main results on studies of ruminant digestive physiology after five decades, using the Cuban Journal of Agricultural Science as the main information source.

Key words: *physiology, digestion, ruminants*

INTRODUCTION

The main characteristic of ruminants is their ability to feed on grasses and forages because they can degrade structural carbohydrates like cellulose, hemicellulose and pectin, which are less digestible for non-ruminant species. Regarding this essential difference, digestive physiology of ruminants acquires particular characteristics because food degradation is mainly performed by fermentative digestion, not by the action of digestive enzymes, and fermentative processes take place due to different types of microorganisms that stay in the stomach cavities of ruminants.

For five decades, the department of Physiology from the Instituto de Ciencia Animal has carried out basic researches that include the different feeding systems used, as well as the way of increasing the efficient use of foods with a correct management of the rumen.

So, after 1965, moment in which the institute was created, researches started and began to explain physiological responses of ruminants in different conventional and alternative feeding systems. These studies comprehend the use and management of grasses, forages and concentrated food, and the application of biotechnology for transforming and increasing their nutritional value.

SUGAR CANE MOLASSES AS AN ENERGY FOOD FOR RUMINANTS

Sugar cane and its products are one of the main alternative sources of feeding for ruminants. Its effects on ruminant digestive physiology constitute the study object of the first researches developed with the use of molasses. Results of Preston *et al.* (1968) demonstrated the influence of different levels of urea on final molasses provided at will, as a supplement for grains during the process of fattening bulls. These authors found that daily ingestion of molasses decreased, and total efficient use of diets increased, while the level of urea increased too, from 3 to 9 %.

Animals with urea had more muscle and less fat in the cut of the tenth rib, and ruminal ammonia concentration increased with the urea percentage in molasses. Besides, pH, SCFA and bacteria and protozoa counting were constant. These studies also demonstrated that minimum protein requirements for cattle fattening, stated by ARC (1965) were very low, and molasses intake decreased with urea levels over 3 %. Since then, only 3 % of urea was applied on molasses for cattle fattening in Cuba.

Later studies tried different types of molasses and established differences between the use of final molasses and high-test molasses. Unfavorable results of high-test molasses were caused by a mineral unbalance, inherent in phosphorous deficiency. However, there were no differences regarding the characteristics of the ruminal content (Elías *et al.* 1967).

Later, the most suitable concentrations of molasses for the different stages of cattle growing and fattening were established, in order to avoid toxicity due to the high intake of this product, and recommend that, for intensive fattening with molasses, it should start between 15 to 35 ºBrix and concentrate it at 75 ºBrix, after the first six weeks (Preston *et al.* 1968).

Elías *et al.* (1969) were the first to establish the optimal inclusion of forages on fattening systems based on urea molasses. These authors pointed out that it should not exceed 0.23 kg MS/100 kg of liveweight, level in which the best conversion of metabolizable energy, as well as the highest concentration of protozoa at ruminal level, were obtained.

Elías and Preston (1969) studied the racial differences in the percentual proportion of ruminal microorganisms in these feeding system, and it is stated that *B. indicus* is a less appropriate breed than *B. taurus* for the intensive feeding systems that use diets with high energy level.

At the same time of these previous studies, researches on the flow of digesta through the gastrointestinal tract began with bulls in feeding systems with urea molasses, where Geerken and Sutherland (1969) found that the ruminal volume is around 20 % of liveweight, and that soluble carbohydrates are also fermented in this organ during their flow through the gastrointestinal tract. A similar result was obtained by Marty and Sutherland (1970), who studied the metabolism of sucrose and lactic acid in the rumen during the adaptation to a diet rich in molasses, and inferred that the constant rate of conversion of sucrose into lactate increases more proportionally than the initial constant rate of sucrose disappearing. The increase of the constant of lactate disappearing was retarded until the last two weeks after the adaptation period. Mean ruminal concentrations of lactic acid and soluble carbohydrates were lower during the intake of diets based on forages.

Marty and Preston (1970), who continued researching on this subject, determined molar proportions of short chain fatty acids (SCFA), produced in the rumen of cattle fed with diets rich in molasses.

The first studies on metabolism of nitrogen and carbohydrates in the rumen, with diets based on molasses, were developed by Kowalczyk *et al.* (1970) in calves with simple cannulas in rumen, reentering in the duodenum. These authors established that the duodenal and ruminal flow, in this category, were 54 and 51 L/d, respectively, and the bacterial N that goes through the rumen and duodenum was 24 and 13.6 g/d, respectively.

Ramírez and Kowalczyk (1971) also estimated microbial protein synthesis in young bulls, fed with diets based on molasses/urea free of true protein, and stated that the amount of N, of synthesized bacterial origin in the rumen and goes through the duodenum, was 25 g/d, which corresponds to 2.5 g of total microbial N per each 100 g of easily fermentable carbohydrates. Ramírez (1972) demonstrated that the deaminating activity of ruminal bacteria decreases when molasses diets are used.

In order to study the total flow of bull saliva with final molasses diets, Benavides *et al.*(1971) introduced a technique for the establishment of a re-entering cannula in the esophagus of bulls from 62 to 104 kg of weight, which allows the measuring of the total saliva flow for 24 h, with liquid diets based on molasses. This way, some physiological aspects of digestion and liquid turnover in the rumen can be better understood, as well as the regulation of food intake and certain aspects related to etiology of metabolic changes like bloat and ruminal dysfunction of nutritional origin.

As a response to the questions that emerged with the intensive use of molasses in cattle, Marty (1971) performed the first attempts to manipulate ruminal fermentation. This author researched with defaunated and non-defaunated animals, in order to study the effect of fermentation on SCFA molar proportion, and relates the presence of butyric acid to the concentration of alcohol produced by molasses. Simultaneously, there were studies on the intoxication due to molasses that lead to the appearing of cerebrocortical necrosis (CCN), as the main cause of diseases produced by this type of food. Lozada and Preston (1973) carried out several experiments to examine the relation between the CCN and some aspects of the metabolism of thiamin. These authors concluded that in CCN, caused by molasses toxicity, brain damage is not motivated by the inability to metabolize piruvate through thiamin deficiency, as in the typical cases of CCN, but by a glucose insufficiency.

In 1974, these authors demonstrated that toxicity was caused by changes in the ruminal fermentation pattern, as a consequence of the decrease in voluntary intake of induced DM, and by the lack of forage in the diet.

Another important physiological study was carried out by Perón and Preston (1971), who established the importance of forage in the development of ruminal wall structure and weights. These authors noticed the danger of parakeratosis and the poor development of ruminal papillae with diets based on molasses. They also indicated that the highest liquid volume in animals only fed with molasses shows a reduction on the ratio of ruminal liquor, as a result of the lack of mechanical stimulation in the ruminal wall.

Marty (1972) states a detailed study about the influence of different factors on the use of food by the ruminants and the real possibilities of ruminal fermentation manipulation for the benefit of host animals and humans.

Reyes (1973) performed *in vivo* and *in vitro* studies on fermentation indicators in the rumen and caeca of bovines, fed with diets based on forage and molasses. These studies demonstrated that these diets establish an specific type of fermentation, which is demonstrated through a relatively high pH, low concentration of VFA, and a decrease of molar percent of acetic and propionic acids, with an increase of butyric acid in the rumen. In this regard, Marty and Henderickx (1973) showed that molasses-rich diets do not have the buffer characteristics, and its effect on pH is caused by its constant ingestion.

The first studies on gas production (Marty *et al.* 1973) suggested an inverse relation between methane production and propionate molar proportions on ruminal liquor of sheep with diets based on molasses.

Ruiz and Molina (1976), after studying the effect of diet on the histochemical performance of some respiratory enzymes, and of the ATPase on the ruminal epithelium of cattle, stated that it was possible that the order of enzymatic activity levels (concentrates>molasses>forages) was related to equivalent absorptive and metabolic activities in the ruminal wall.

Ruiz (1989) verified these results by measuring the effects of intake of high levels of molasses on the structure and function of ruminal wall of bulls. Histopathological study showed that the main change found in the ruminal mucus of animals fed with final molasses of sugar was the keratinization of the corneous stratum with retention of nucleus. The epithelium seemed decreased, and the cells of the lucid stratum were narrowed in the spindle-shaped cores. The grain stratum was not different from the thorny one. The thickness of corneous stratum was not homogeneous and there were zones with thick layers of corneous substances. The intense dark color of ruminal epithelium was attributed to the high content of iron, produced by the constant intake of molasses.

Chongo and Thivend (1982) found deleterious effects on carbohydrate digestion, after using final molasses on lactating calves.

OTHER PRODUCTS OF SUGAR CANE

Residues from sugar cane cleaning centers, as an alternative food for ruminants, were also researched during the 90´s. Fundora *et al.* (1992, 1993) evaluated the increase of digestibility and minerals and N balance in several categories of ruminants, when sugar cane harvesting residues were treated with ammonia. Delgado *et al.* (1993) and Galindo *et al.* (1993) demonstrated previous researches by stating that residues treated with ammonia increased intake and digestibility of dry matter and N, and, at the same time, offered a proper proportion of ammonia N to ruminal microorganisms, which have the function of digesting fibrous foods, due to the increase of cellulolytic bacteria population.

Other results with sugar cane products were obtained with the use of sugar cane juice as a supplement for dairy cows in the ruminal microbiology and metabolic and physiological aspects. Gutiérrez *et al.* (2005a) demonstrated that the inclusion of fresh sugar cane juice on systems based on low quality grasses did not affect intake and digestibility of nutrients, acid-basic balance indicator nor the metabolic profile of dairy cows.

Once stated the advantages, disadvantages and risks of ruminant feeding systems with high levels of molasses, in almost total absence of forage and other sugar cane products, digestive physiology studies were focused towards the best use of grasses and forages with supplementation of energy, protein and mineral foods, in the necessary cases. Those facts, plus the state policy of achieving the maximum exploitation of fiber foods for ruminants, became a new alternative for ruminant physiology studies

GRASSES AND FORAGES AS BASIC DIETS FOR RUMINANTS. ADDITIVES TO INCREASE EFFICIENCY USE OF NUTRIENTS

The first studies to describe the effects of fibrous diets on digestive physiology were performed by Geerken *et al.* (1977) and Martín *et al.* (1977). These authors studied the metabolism of energy and N in calves fed with forages of Coast cross No.1 Bermuda grass (*Cynodon dactylon*) and pangola grass (*Digitaria decumbens* Stent), and the effect of NaOH level on *in vitro* production of total short chain fatty acids (AGCC) of treated bagasse pith and bagasse.

The nutritional advantages of Coast cross No.1 bermuda grass, compared to pangola grass, were demonstrated due to a higher retention of nitrogen and energy in animals. These authors also demonstrated the beneficial effect of NaOH in the structural carbohydrates of bagasse pith because it favors a higher utilization by ruminal microorganisms and, consequently, a high level of final products from the substratum fermentation.

After using grasses, Geerken *et al.* (1980) studied the effect of N supplementation on digestibility and intake of Coast cross No.1 bermuda grass (*Cynodon dactylon*) in calves. These authors reported that, in grasses of medium quality, N supplementation contributed to increase nutrient digestibility and intake because its primary action started on the activity of ruminal microorganisms.

Gutiérrez *et al.* (1980a) defined the chemical structure and availability of phosphorous, contained in bermuda and pangola grasses, and demonstrated that this element was an inorganic compound with high biological availability for ruminants. These authors also studied the intermediary metabolism with the use of 32p, in order to establish the true digestibility of phosphorous, contained in Coast cross No.1 bermuda grass, in growing calves, and demonstrated that it was superior to 85 %. Gutiérrez *et al.* (1980b) studied grazing animals in phosphorous deficient areas and pointed out the possibility of identifying the deficiencies through the study of metabolic profile.

The studies on metabolism of nitrogen compounds in ruminants with grasses and forages were started by Coto *et al.* (1981). These researchers presented the first results of the flow of N compounds towards the duodenum in cows consuming Coast cross No.1 bermuda grass, and demonstrated that the amino acid proportion that reaches the duodenum was similar to the microbial protein, with the exception of the glycine proportion because it was high. These authors also identified the rate of microbial synthesis in the rumen with Coast cross No.1 bermuda grass under *in vitro* conditions.

ADDITIVES FOR IMPROVING FOOD EFFICIENCY OF BAD QUALITY FORAGES

Galindo *et al.* (1982, 1984 and 1990) continued the studies related to the use of additives for improving efficient use of nutrients in fiber-rich diets. These authors verified that natural zeolite worked as improver of nutritional values of silages from bad quality grasses because this mineral was able of increasing ruminal cellulose degradation, when it was added to 1 % of the diet. Zeolite also increased the activity of the enzyme-cellulase complex, and improved the microbial balance and the indicators of ruminal environment in cows consuming silages as basal diet.

Gutiérrez *et al.* (1983) demonstrated the biological availability of phosphorous contained in a national superphosphate, which was used as additive in diets for growing calves. Further studies reported the increase of nutrient digestibility when calcium phosphate was added to the diet.

Gutiérrez (2010) researched the influence of national or international mineral sources in ruminant digestive physiology and metabolism, and demonstrated that calcium and phosphorous, from Cuban phosphoric rock, respond to the hydroxyapatite structure, where fluorine is mainly found as calcium fluoride.

Phosphate limestone has similar characteristics to importation limestone, due to its buffer action for dairy cows, when high concentrations are used in the diet. In addition, minerals contained in national iron, copper, cobalt and manganese sulfates are biologically available for ruminants, and natural zeolites have no buffering action but regulate the internal medium due to their ability of cationic exchange. Betonite protects the dietetic protein from the attack of ruminal microorganisms.

In order to know the effects of adding non-protein N sources on nutrient digestibility of animals fed with different grasses, Muñoz *et al.* (1984) carried out studies that allowed them to verify the increases in the use of nutrients and the high acceptability of this type of supplement. These results were confirmed by researches of Galindo *et al.* (1996), who reported that, in diets of integral sugar cane, nitrogen activating supplements of ruminal fermentation favor ruminal ecology and provide better productive results.

Coto *et al.* (1985) also measured the rate of microbial synthesis in the rumen, using the *in vitro* technique and 32p as indicator, and reported that low concentrate levels increased microbial synthesis.

In other study, Gutiérrez *et al.* (2005a) demonstrated the increase of intake and digestibility of dry matter in cows with pastures and protein banks (*Leucaena leucocephala*).

Ionophore are other additives for improving efficient use of fibrous foods. In this sense, it was also important the use of plants able of reducing methane production and, consequently, the energy economy. Galindo *et al.* (2003) used the monensín in several ruminal ecological systems, and demonstrated that the addition of this ionophore reduces ruminal methanogenesis, although it should be used with precaution if the maximum degradation of cellulose is wanted in the rumen, because it selectively decreases the cellulolytic bacteria population.

González *et al.* (2006) used the PCR technique in real time and *in vitro* gas production for measuring the effects of the bromine-ethane-sulfonic acid on methanogenesis and on ruminal microbial population. These authors concluded that this product had a great influence on inhibition of ruminal methane production, without changing the populations that degrade the fiber.

BIOTRANSFORMED FOODS AND THE INCREASE OF THEIR NUTRITIONAL VALUES

During the 90´s, a new stage starts in the use of sugar cane. Elías *et al.* (1990) proposes a new product based on the fermentation of solid state of clean and ground sugar cane, with the addition of urea and minerals, where soluble carbohydrates decrease and non-protein nitrogen (NPN) are transformed into precipitate nitrogen to triclhoroacetic acid. This new product was called "Saccharina", and constituted the main cattle food source, which was an object of later studies of digestive physiology in ruminants during this period.

After obtaining this product, many researches were developed, which included from the most suitable size of particle up to digestibility and nutrient passage through the gastrointestinal tract of ruminants and its influence on ruminal environment. Geerken *et al.* (1994) demonstrated that particle size of chopped or ground Saccharina, provided at 65 % of the diet, had no negative influence on the intake or on food performance of grown sheep. However, chewing effectiveness was increased up to reaching the proper particle size for its swallowing and passing through the gastrointestinal tract (Delgado *et al.* 1997).

Ruiz *et al.* (1990), after analyzing the effects of substituting feedstuff cereals for Saccharina in the diet of sheep fed with hay as basal diet, found that digestibility of DM, N and OM was not different among experimental treatments, apart from the level of Saccharina included on the concentrate. However, there was an increase of CF digestibility ($P < 0.001$) with its inclusion level.

In some other researches, Delgado *et al.* (1991) and Gutiérrez *et al.* (1992) measured the passage of nutrients towards low parts of the tract, and the rechange of ruminal liquor in cows consuming Saccharina within the feedstuff. These authors concluded that up to 90 % of substitution levels do not change the passage of nutrients to the duodenum. Besides, these researches demonstrated that the use of this product increased the efficiency of nitrogen compounds and of higher minerals, and pointed out the transformation of inorganic phosphorous into organic phosphate, as a constituent of the synthesized microbial cell. They also found a significant increase of the specific activity of the enzyme-cellulase complex and the microbial net yield, which was measured as synthesized microbial cell grams/mol of fermented ATP (YATP), as well as the moderate efficiency of microbial synthesis in all evaluated diets (Galindo *et al.* 1996).

Another product obtained through biotechnology was protein molasses. This product was achieved through fermentation, in liquid state, of sugar cane B molasses with urea and mineral sources. This process is highly aerobic and a microbial product is obtained from it, which contains approximately from 16 to 22 % of CP, from 7 to 12 % of TP, and 10^7 cfu/mL of living yeasts.

Although most of the researches with protein molasses were carried out with non-ruminant species, Galindo *et al.* (2005) showed that it was feasible to supply 4.5 kg of protein molasses and 100 g of urea to cows consuming king grass and sugar cane forage as basal diet, because the total viable bacteria population increases, as well as the ruminal cellulitic activity. This can produce a higher degradation of the fiber fraction, and, consequently, productive improvements.

TREES AND SHRUBS AS A COMPLEMENT OF RUMINANT FEEDING

At the end of the 90´s, new researches on ruminant digestive physiology began, which were related to the potential value of tropical areas as food provider, because these areas have a biological diversity of plants able of complementing diets as a protein source, or of manipulating fermentative processes at ruminal level.

Leucaena leucocephala, due to its nutritional value, could be compared to alfalfa or cajanus. Nevertheless, the presence of mimosine, a secondary metabolite, led to several researches on its possible degradation by ruminal microorganisms. Galindo *et al.* (1995) described the effects of leucaena on the ruminal ecosystem of cows consuming king grass and sugar cane forage. These authors found that *Leucaena leucocephala* improves the composition of cellulitic microorganisms. Therefore, it favors a higher ruminal cellulose degradation, and offers the animals the amount of sugars necessary for their metabolism. These studies also demonstrate that there are bacteria able of degrading mimosine, as well as its highly toxic hydrolysis products (3-hydroxi-4(1H)-pyridone (3, 4-DHP)), within the rumen, under the edaphoclimatic conditions of Cuba.

Researches of Delgado *et al.* (1996) demonstrated that the supplementation with leucaena improved total fiber digestibility and increased the intake of dry matter up to the level that depends on NDF concentration in the rumen of sheep fed with hay of star grass. La O *et al.* (2003, 2012) confirmed those results when they stated the high ability of several ecotypes of leucaena and *Tithonia diversifolia* to provide degradable protein to the rumen and increase its use in feeding ruminants.

Enterolobium cyclocarpum, *Sapindus saponaria* and *Gliricidia sepium* contain high concentrations of proteins and low amount of fiber, compared to grasses, which allows their use as a supplement for balancing diets of grasses and forages of low nutritional quality. *Enterolobium cyclocarpum* increases total microbial population, as well as the amount of cellulolitic organisms, while the *Gliricidia sepium* produces defaunating effects and increases the population of cellulolytic organisms, so it favors the degradation of fibrous materials in the rumen (Delgado *et al.* 2001 and Galindo *et al.* 2001ab).

Delgado *et al.* (2002) studied new varieties and stated that *Brosimum alicastrum* and *Bauhinia galpinii* have good nutritional quality with more than 60 % of dry matter degradability. In addition, Galindo *et al.* (2003) demonstrated that *Brosimum alicastrum*r reduces the population of total viable and proteolytic microorganisms in the rumen, which can be beneficial for the protection of dietary protein at this proportion.

González *et al.* (2012) and Galindo *et al.* (2011) evaluated the inclusion of 30 % of different varieties of mulberry (*Morus alba* L.) on the production of ruminal methane. These authors informed that the Cuban variety was the most promising at this level of inclusion, while *Tithonia diversifolia* reduced the protozoa population and ruminal methanogens under *in vitro* conditions. The inclusion of 27 % of *L. leucocephala* on a basal diet of *P. purpureum* increased DM and OM intake, and the methane production was reduced in 15.6 %, in L/kg of consumed DM, without affecting apparent digestibility of nutrients in ovine (Delgado *et al.* 2013).

Delgado *et al.* (2009) and Galindo *et al.* (2012) studied a group of phytogenetic resources and demonstrated that tropical plants like *Samanea saman*, *Albizia lebbeck*, *Azadirachta indica*, *Tithonia diversifolia* plant material 23, *Cordia alba*, *Leucaena leucocephala*, *Pithecelobium dulce*, *Moringa oleifera*, *Gliricidia sepium*, *Guazuma ulmifolia*, *Tithonia diversifolia* plant material 10 and *Enterolobium cyclocarpum* can be used for reducing protozoa population and ruminal methanogenesis.

Benefits of phytogenetic resources in food complementation of ruminants, as well as its contribution to the reduction of methanogenesis, are still under study because of their great importance.

DIGESTIVE PHYSIOLOGY OF BUFFALOES COMPARED TO CATTLE

Buffaloes were introduced in Cuba in 1983 and were located in almost every area for cattle rearing that were not appropriate for bovine development. This started a new period of basic researches related to digestive physiology, metabolic profile and voluntary intake of this new species.

In order to study digestive physiology, metabolic profile and ruminal microbiology of water buffaloes, an experimental sequence was performed by Rodríguez *et al.* (2003), Delgado *et al.* (2005), Delgado y Cairo (2008), Valenciaga *et al.* (2007, 2008), Cardentey *et al.* (2008) and González *et al.* (2012).

The researches of these authors showed that the increase of energy level in buffaloes ration from 63 to 98 MJ. kg^{-1} DM had no significant changes on the population of ruminal cellulolitic, proteolytic and aminolytic bacteria, and cellulolitic fungi. It had no significant effects on total degradability of forage, but there was a decrease of pH and an increase of molar proportions of SCFA, as a consequence of the inclusion of easily fermented materials on the ration. These energy values did not modify the concentration of $N\text{-}NH_3$ and were over that considered as a limiting for digestion (5 to 10mg N. 100 mL^{-1}) or intake (5 to 8mg N. 100 mL^{-1}).

After studying three protein levels for covering 100, 115 and 130 % of the protein requirements of bovines and buffaloes, there were no effects of N supplementation on the ruminal microbial population, fermentation pattern, ammonia N of the rumen and pH. However, the increase of protein level up to 30 %, over the maintenance requirements, in the diet provoked increases in the intake of DM, NDF, ADF and CP.

Total intake of DM ranged between 2.1 and 2.3% of LW, while the supplementation favored apparent digestibility of protein, but this value was inferior in buffaloes, compared to cattle

When comparing ruminal microbial populations of Zebu bulls and river buffaloes, cellulolytic and total viable bacteria were not different between both species, apart from the diet they consumed. Proteolytic bacteria population was three times superior in buffaloes, regarding Zebu cattle. This demonstrated that buffaloes can develop a better ability for degrading proteins than cattle. There was a faster colonization of fiber by ruminal microorganisms of buffaloes, which is important for the total digestion of fibrous materials, depending on the ruminal passage towards inferior parts of the gastrointestinal tract.

Buffaloes had a lower representation of protozoa from Diplodinium compared to Zebu cattle and there were no differences for Entodinium, Eodinium, Diploplastron, Eudiplodinium, Polyplastron, Epidinium, Isotricha, Dasytricha and Ophryoscolex genera. The largest populations of the Diplodiniinae subfamily were observed in the ruminal liquor of buffaloes. These results demonstrated that, when hosts feed on the same type of ration and they are located in the same area, they can show the same protozoa genera.

There were no differences in the *in situ* degradability of DM of grasses and legumes in different feeding systems between bovine and buffaloes. However, using some forages, buffaloes reached a higher degradability of fiber material during the first 12-24 hours of stay in the rumen.

Buffaloes are able of using a basal diet of sugar cane/urea integral forage and a supplementation with fresh forage and soybean meal, similar to Zebu cattle. They are also able of using more efficiently the available energy of sugar cane because they keep high ruminal pH (6.4-6.9), regarding that of bovine (6.1-6.4) and have higher concentration of total SCFA and acetic acid in the rumen. Buffaloes had higher acetic: propionic relation than bovines.

The contribution of 28 or 42 % of crude protein in the ration, like non degradable nitrogen in the rumen (NDNR) given by raw or extruded soybean or in the concentrate for buffaloes produced appropriate values of pH (6.55 and 6.79, respectively). The use of extruded soybean increased apparent degradability of crude protein regarding crude soybean (63.45 vs. 47.16 %), while the excretion of allantoin was 41.19 and 40.48 mmoles. day^{-1}, respectively, which represented 86 and 89 % of all the excreted purine derivates (PD), and the excretion of purine derivates was not affected. Average synthesis of microbial nitrogen was 33 g. day^{-1}.

The comparison of ruminal microbial degradability of *Pennisetum purpureum* cv. CUBA CT –115 between buffaloes and Zebu cattle demonstrated that the kinetics of DM degradation and of the constituents of the cell wall was similar in both species. There was a fast increase of nutrient degradation with time of incubation up to around 24 hours, and later the increase was slow, almost constant up to 72 hours. However, ruminal effective degradability (RED) in buffaloes, regarding Zebu cattle, was 38.82 vs. 34.58%; 33.94 vs. 29.15%; 25.96 vs. 22.04%; 42.84 vs. 38.27% and 39.25 vs. 35.45% for DM, NDF, ADF, cellulose and hemicellulose, respectively.

Likewise, buffaloes reached higher rates of ruminal degradation and their period of latency or lag phase of fermentation was inferior to that of Zebu cattle (5.02 vs. 7.51 h, 5.22 vs. 7.69 h, 6.17 vs. 8.26 h and 5.58 vs. 8.22 h for NDF, ADF, cellulose and hemicellulose, respectively). Results suggest the best ruminal efficient use of *Pennisetum purpureum* cv. CUBA CT-115 with 140 days of regrowth in river buffaloes, compared to Zebu cattle.

When studying the digestive use of foliage of *Gliricidia sepium* and *Leucaena leucocephala* of buffaloes, researchers found that this species has high potential of ruminal degradation of DM, with values superior to 80% in gliricidia and 77% in leucaena. The potentially degradable fraction (B) and potential degradability of leucaena were higher in buffaloes, regarding bovines, while gliricidia was equally degraded by both species.

Potential degradability of N from gliricidia was near 94 % in buffaloes, and 89% in bovines. Rate of DM degradation of gliricidia was 6 %. h^{-1}, similar between buffaloes and bovines, but leucaena showed a degradation rate of 7 %. h^{-1} in Zebu, while buffaloes had 4.8 %. Likewise, the evaluation of four mulberry (*Morus alba*) varieties evidenced that those varieties increased ruminal fermentative ability, which makes possible the use of this source as a supplement in daily ration for buffaloes.

Metabolic profile of female buffaloes showed that serum values of total proteins, albumen, iron and magnesium increased during the last third of gestation. Hemoglobin and, specially, plasmatic proteins could indicate the presence of any factor of metabolic risk, because they express the low protein contribution in the ration consumed by female buffaloes. Researchers stated that milk production did not provoke changes in the values of studied hematochemical indexes, and the mean values of serum proteins were lower under conditions of low inputs than those reported in superior levels of feeding.

Gutiérrez *et al.* (2014) studied voluntary intake and nutrient digestibility of female buffaloes in grazing (low quality grasses) and showed positive effects of mineral supplementation on productive and reproductive indexes of this species.

FINAL CONSIDERATIONS

The integral analysis of this review shows the intense work carried out for fifty years, in order to distinguish the main issues of digestive physiology of ruminants when they are located in different feeding systems. These basic studies are essential when the aim is to manipulate the diet as to obtain the best and most important responses from the productive and reproductive point of view of this species.

Basic researches related to digestive physiology of ruminants clarified a considerable amount of questions related to, for instance, the use of final molasses that constituted the main food base for bovine fattening in the country for a long time. In this sense, the causes of cerebrocortical necrosis were described because they were one of the first economic losses from the use of this energy source. The importance of fiber diets, in the adequate functioning of the rumen and the specific characteristics of its wall for the indiscriminate use of molasses, were used as bases for establishing the optimal inclusion level of forages in the proposed system.

There is an important amount of information that demonstrates the physiological effects of the use of additives for complementing the nutritional value of grasses and forages, combining the studies of ruminal microbiology with other aspects of intermediary metabolism of different ruminant species.

There is no doubt on the importance of bio-transformation of food with low protein values and their influence on ruminant digestive physiology, which was clearly stated in this review.

This review also indicates the real possibility of using several phytogenetic resources as a complement for ruminant diets, avoiding their inclusion level to affect ruminant digestive physiology due to the presence of secondary metabolites that could be transformed or not by the rumen.

Finally, there is detailed information about digestive physiology of buffaloes as a recently introduced species in Cuba.

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