v. 4, n. 8, 2024

Journal of Agricultural Sciences Research

PHYSIOLOGICAL CHANGES PRODUCED BY THE BETA ADRENERGIC AGONIST-CLEMBUTEROL-β2AA-CLB, IN BOVINE

Ricardo E. Caicedo Rivas
Doctorate in Physiology and Comparative Endocrinology, ``Universidad de Gifu``-College of Agriculture-Rengo Daigaku-Gifu, Japan. Faculty of Biological Sciences/ Experimental Morphophysiology Area/ Senior Research Professor; ``Benemérita Universidad Autónoma de Puebla``-BUAP, Faculty of Biological Sciences, Laboratory of Reproductive Endocrinology and Malacology Puebla, Mexico

José. A. Contreras Salazar
Student of the degree in Biology, Faculty of Biological Sciences, ``Benemérita Universidad Autónoma de Puebla`` Faculty of Biological Sciences, Laboratory of Reproductive Endocrinology and Malacology Puebla, Mexico

Janeth Escobar San Martin
Student of the degree in Biology, Faculty of Biological Sciences, ``Benemérita Universidad Autónoma de Puebla`` Faculty of Biological Sciences, Laboratory of Reproductive Endocrinology and Malacology Puebla, Mexico

Mariana Paz-Calderón Nieto
Master in Productive Biology, at the Applied Biology Research Center - ``Instituto Politécnico Nacional``-IPN
Bachelor's degree in Biology ``Benemérita Universidad Autónoma de Puebla``-BUAP, Biological Sciences Faculty, Professor of Biology and Biochemistry at the Regional Center of the Complex, Amozoc, ``Benemérita Universidad Autónoma de Puebla``, Mexico
Faculty of Biological Sciences, Laboratory of Reproductive Endocrinology and Malacology Puebla, Mexico

Abstract: Nowadays, there are several latent problems worldwide linked to food production: 1- the increase in world population, 2- the increase in war conflicts, the latter linked to the distribution of raw materials for food production, and finally 3- global climate changes, however, biotechnological processes for food production influence the increase in the production of synthetic foods and the use of food additives in animals, their products and by-products are consumed by man, All this in order to increase foods with high nutritional and energy value, which do not nourish, bringing with them an increase in diseases to the animal itself and to man, for this this research has been developed, carried out for more than 15 years of work, which today shows that the use of feed additives is increasing in many livestock regions, mainly in feed a synthetic component called Beta-adrenergic agonist (β2AA), such as clenbuterol-Clb. This is considered a powerful broncho-vessel-dilator, used to control respiratory diseases in horses and humans mainly; Clb is a β2-adrenergic agonist (β2-AA), which causes an increase in muscle mass in birds, cattle, goats and sheep. On the other hand, the metabolic pathways on the effect of Clb are not known to date. They are well elucidated; The objective of this study was to determine the metabolites that arise with the ingestion of clenbuterol-Clb and its possible derivatives that are stored mainly in the liver, pancreatic structure, adrenal gland, smooth and striated muscles, central and peripheral nervous system and genital organs. The fat content is dramatically reduced when clenbuterol is used as anabolic, understood as anabolic, when Clb is administered orally or intramuscularly above the therapeutic dose, between 5 and 10 times its concentration (0.08μg/body weight two times a day). For this study, 17 different metabolites were measured with the purpose of determining which

metabolic parameters are altered, producing a homeostatic imbalance at the level of the different organs in which it intervenes. The results showed that there are several metabolites that are altered by clenbuterol-Clb, among these we have: glucose, triglycerides, alkaline phosphatase, Gamma Glutamyl Transferase, transaminases (ALT/GPT and AST/GOT), Lactic Dehydrogenase, Prostatic Acid Phosphatase and not Prostate, cholesterol and calcium. The changes produced mainly in the blood metabolites present in each animal, indicated that it has an effect of changing the metabolism of various organs and affects their metabolic pathways, contributing to its lipolytic and antilipogenic activity and induces nitrogen retention, increases the Glycolysis, the production of lactate, increases the animal's body temperature and oxygen consumption, increases glucose levels, which varies according to the time of treatment with Clb, since insulin and adipocytes decrease at the level of the pancreas. are less sensitive to this hormone, there is an increase in energy use, bringing with it an increase in body temperature-thermogenesis, decreasing spermatogenesis and altering sperm morphology, it was even detected in ovarian follicles. The most significant damage occurs at the level of the liver parenchyma, producing hepatomegaly, liver tumors, possibly granulomatous hepatitis and finally cirrhosis, however, the anomalies are not detected at the phenotypic level, but by measuring liver enzymes, likewise, Cases of human poisoning have also been detected, from 2002 to date, in 2023, there has already been one death due to the consumption of this food additive, through beef. In conclusion, the data provide evidence of the damage that Clb produces at the level of the homeostatic process in ruminants. Furthermore, due to its biochemical characteristics of Clb, its bioavailability will depend on the time it was administered and the biotransformation will depend on the concentration of Clb with which it is administered. the animal was fed. On the other hand, liver damage is irreparable.
Keywords: Metabolic pathways, Bioavailability, Biotransformation, metabolic profile, metabolic pathways

INTRODUCTION

The use of β2-agonists, mainly clenbuterol (Clb), zilpaterol and ractopamine, is producing an increase in poisoning in humans and animals, mainly due to the excessive use of these components that are used as food additives (Sumano et al., 2002, Caicedo et al., 2009, 2011, Valladares et al., 2013 and Caicedo et al., 2021).

On the other hand, other components have also been developed that improve the body quality of animals such as: antibiotics, prebiotics, enzymes, antimicrobials, immune system modifiers, metabolic modifiers or anabolic agents. In recent years, the use of β2-adrenergic agonists (β2-AA) has increased in animals of economic importance; consider that the excessive use of this drug such as clenbuterol (Clb) has had and continues to have a very significant toxic impact on humans and animals (altering animal and human well-being, respectively). Its use increases meat production in the short term, in three months the live weight of the animal can be increased by 50-80%, this will depend on the concentration of Clb that is supplied to the animal, as well as the administration time (Caicedo et al., 2021), since the Clb tends to retain nitrogenous compounds, increasing muscle mass (Smith, 1998). β2-AA agonists increase degradative lipid metabolism in adipocytes in vitro and in vivo. In mammalian tissues, there are up to moment three different subtypes of βAR-receptors of the β2-AA, such as: β1 (β1AR), β2 (β2AR) and β3 (β3AR). Individual tissues (for example: liver, heart) have different proportions

of subtypes, this will depend on the vertebrate species, and it varies between different species. Consequently, certain β2-AA agonists are expected to have different effects in the same tissue in different species due to differences in the distribution of βAR subtypes and (or) amino acid sequence (Mersmann, 2002); For this reason, it can be added that the effects produced by a βAR agonist (or antagonist) in adipose tissue in vivo depend not only on the species and distribution of βAR subtypes of the adipocyte, but also on the pharmacokinetics and pharmacodynamics of the compound in that species, including blood flow to the tissue, and the multiple metabolic and endocrine effects of the compound on others. tissues of the body (Mersmann, 2002); (**Figure 1**).

These β2-AA drugs are also chemical agents that act specifically at the level of cellular adrenergic receptors (β-AR), metabolizing nutrients and increasing energy, increasing protein and fat metabolism, modifying membrane permeability. cellular, increased lipolysis and glycogenolysis (Meyer and Rinke, 1991), due to the location of their receptors (transmembrane hydrophobic domains) in the cell membrane where they act. ***Figure 2***.

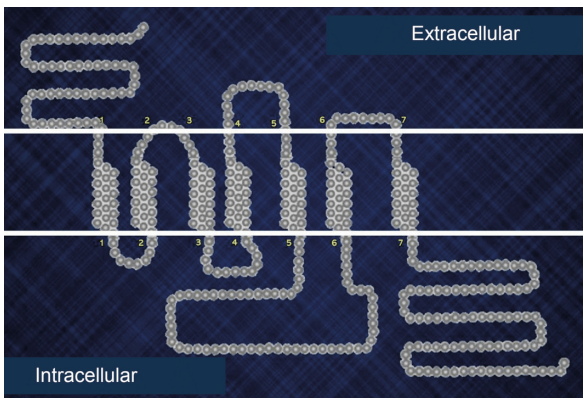

Figure 2: All β-AR-Clb receptors contain seven transmembrane hydrophobic domains (see numbers-1,2,3,4,5,6,7) which allows this component, like Clb, to have a much longer and slower effect, mainly in adipocytes. According to Johnson et al., 2014, (Asian Australasian J. Anim. Sci., Vol.27, No. 5: 757-766)

In addition, they bring with them an increase in the formation of muscle mass, and this is because the OH group that other β2-AA possess, in the case of Clb, is replaced by a halogen called chlorine (Cl), this Cl ion prevents biotransformation by COMT (catechol-O-methyl-transferase) enzymes at the tissue level and hepatic biotransformation is slow (Courtheyn et al., 1996), this chloride ion in clenbuterol makes it more fat-soluble than its analogues (zilpaterol, salbutamol and ractopamine) and as a result tends to diffuse more deeply into tissues and animal fat (Martin 1971, Ruffolo, 1991, Waldeck and Widmark, 1995). ***Figure 3***.

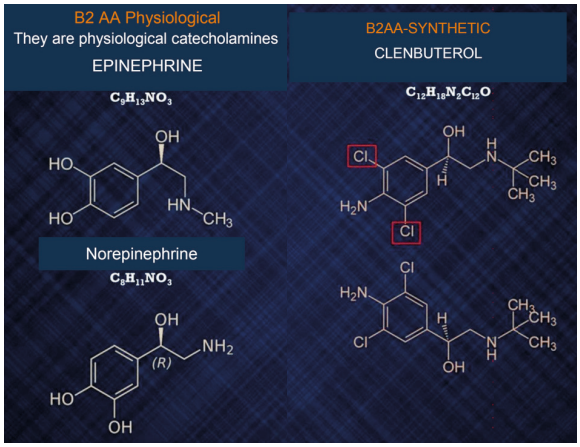

Figure 3: Comparison of the chemical structure of three components called phenethanolamines, two produced physiologically by the body called: Epinephrine and Norepinephrine (Adrenaline and Norepinephrine), both are hormones of the catecholamine type and the other is a synthetic component called clenbuterol-Clb, this is It is characterized by having two chlorine-Cl atoms in its structure, which gives it a prolonged action through the cell membrane and allows it to act very slowly, avoiding its rapid degradation. (Taken from Valladares et al., 2015).

The application of β2-AA to mammals amplifies weight gain, this is possibly due to the increase in the amount of tRNA (transfer ribonucleic acid) for several skeletal muscle

DISTRIBUTION OF MAMMAL B-AR RECEPTOR SUBTYPES

Species	Tissue / Organ	Subtype abundance (B-AR)
Human	lung	27% β_1
	liver	20% β2
	adipose tissue	35% β1----65% β2
Cattle	skeletal muscle	>99% β2
	adipose tissue	>90% β2

Figure 1: The distribution of β-AR receptor subtypes in mammals is presented where Beta-1 (β1): are located in the kidney, adipocytes and heart, their action increases cardiac activity, increases the release of renin and also increases lipolysis and with postsynaptic activity, while Beta-receptors 2 (β2): are located in the bronchial smooth muscle, in the vascular uterine bed, skeletal muscle, liver, pancreas, adipocytes, respiratory smooth muscle and their action is to increase vasodilation, bronchodilation, uterine and bladder relaxation, as well as stimulate gastrointestinal relaxation, increases lipolysis and glycogenolysis hepatic, its action is presynaptic and postsynaptic, finally the Beta-3 (β3) receptors: they are usually located in bronchial and vascular smooth muscle, ganglion tissue, also in adipocytes, their action is to increase lipolysis.

proteins, in this case, after treatment with β2-AA mRNA for light chain myosin (Smith et al., 1998), α-actin mRNA (Helferich et al., 1990) and the protease inhibitor calpain-calpastin (Higgins et al., 1988) are increased.). β2-AA can increase blood flow in certain regions of the body, allowing the process of skeletal muscle hypertrophy by containing greater amounts of substrate and energy sources for protein synthesis. Theoretically, the use of these substances presents a series of advantages related, not only to improving productivity, but also to the quality of the meat, since meat from animals treated with β2-AA has a higher percentage of lean tissue. (Beermann, 1993; Waldeck and Widmark 1995 and Mersmann, 1998). However, the increase in the use of β2-AA is related to the increase in poisoning in humans, according to Kuri, et al (2007); The therapeutic dose (DT) is considered to be 0.8 μg/kg of body weight twice a day.

The maximum duration of treatment in non-lactating cattle allowed is 10 days orally or intravenously. The illegal use of Clb and analogues in livestock is any dose that exceeds the therapeutic dose (Sauer et al, 1995). Based on the above, it is shown that until now the metabolic routes (biotransformation and bioavailability) of degradation of this food additive are unknown, therefore, the objective of this study was to determine the possible metabolic routes of Clb in cattle and its effect. in the homeostatic deterioration of the animal.

MATERIALS AND METHODS

Animals: A total of 3600 cattles (Bos taurus X Bos indicus) were used, a total of 91% were males and 9% females, coming from different zoogeographic zones of the country and from different municipal slaughterhouses and private farms. All the farms under study were georeferenced with a GPS. The ages of the animals ranged from 22-38 months, the predominant breeds: cross breeds: Angus.

Sample collection: Vacuum test tubes were used, the first tube without anticoagulant, to obtain the blood serum for the determination of the metabolic profile and hormonal profile of steroids mainly (progesterone and 17β-estradiol) and another tube with EDTA, for the performing blood smears and thus determining the blood count (the differential count of leukocytes and measurement of hemoglobin). The blood without EDTA was centrifuged at 2,500 rpm/10min, the serum obtained was separated in Eppendorf tubes and frozen at -20ºC for subsequent analysis of blood metabolites, the Bio-System-USA kit was used and 18 metabolites were measured such as : macrominerals: calcium, phosphorus, liver enzymes: Gamma-glutamyl transferase (γGT), lactic dehydrogenase (L-DH), Alkaline phosphatase (FA); transaminases such as: alanine amino-transferase (ALT/GPT) and aspartate amino-transferase (AST/GOT), metabolites such as: albumin, direct and total bilirubin, total cholesterol, Glucose, total proteins, Urea/BUN, prostate enzymes: acid phosphatase total (FAt), non-prostatic and prostatic; Measurements were performed in a spectrophotometer (Spectronic 20).

For the determination of clenbuterol, the RIDASCREEN kit, Clenbuterol Fast (R-Biopharm AG, Darmstadt, Germany) was used, to measure the concentrations of steroids: progesterone (P4) and 17β-estradiol (E2), the immunodiagnosis technique of ELISA (Enzyme-linked immunosorbent assay), USA diagnostic kits were used, they were measured in an ELISA reader (Stat Fax-2100, Microplate Reader).

Statistical Analysis: an analysis of variance (ANOVA) was performed on the data obtained, with the statistical program Stat-2 (Olivares 1984) and to determine the significance between averages, the Duncan New Multiple Range Test was used.

RESULTS

EFFECT OF THE CONCENTRATIONS ADMINISTERED IN CATTLE WITH CLB

The results showed that Clb, (β2-AA) alters the growth and composition of the muscle, apparently, decreasing muscle growth when lipogenesis decreases, stimulates lipolysis; the increase in muscle mass, which is associated with the proliferation of satellite cells, stimulating myofibrils in protein synthesis and suppressing the degradation of myofibrils in protein degradation. The sampled animals increased their body weight between 50 to 70% of their initial weight, in a period of 90 to 112 days of treatment, daily feeding with Clb was twice a day, at a concentration between 5 to 10 times the dose. therapeutic (0.8µg/Kg-body weight), bone at anabolic dose.

A first sampling of bovine animals taken to the slaughterhouse with a slaughter weight between 457±21.6 to 672.8±39.9 kg, in a treatment period between 75.8±8.9 days to 121.7±11.3 days and with ages that fluctuated between 25±1.0 to 38.9±2.2 months, Figure 3 shows the Clb concentrations detected in these animals ready for distribution and sale, the Clb concentrations fluctuated between 112.4±5.9 to 778±12.5 ng/kg. ***Figure 4***

A second sampling carried out 12 months after the first, Clb concentrations were detected in blood tissue, these fluctuated between 245±34.5 to 1623±146.6 ng/kg. If

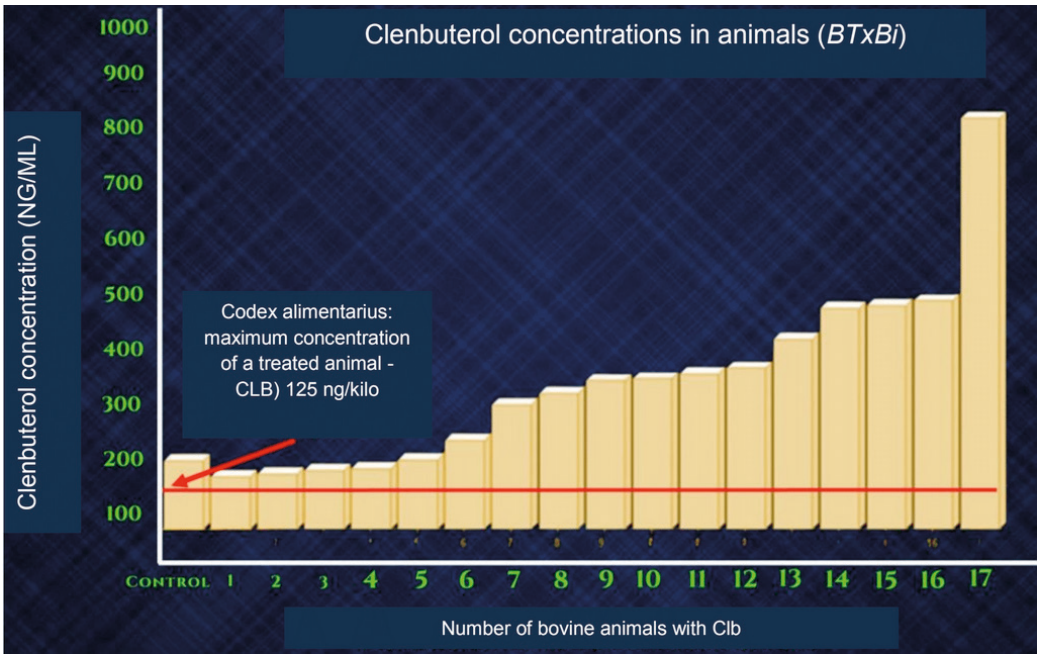

Figure 4: The Clb concentrations of 17 cattle in a slaughterhouse are shown, where the majority of animals were detected with Clb concentrations above those allowed by the Codex alimentarius-FAO (125ng/kg), the red line indicates the maximum concentration allowed. For human consumption.

these values are analyzed, they correspond to bovine animals ready to be distributed in different supermarkets. to be consumed by man, however, these values do not coincide with the values accepted by the FAO/WHO-(Codex Alimentarious), whose values must not exceed 125.0 ng/kg, if the animal has been treated with Clb, and exceeds this value, then it must undergo a quarantine period, so that these values present in the blood decrease through its degradation at the blood and liver level. However, the high concentrations of Clb detected in this study have coincided with cases of poisoning. in humans due to the ingestion of bovine viscera, which is where this β2-AA -Clb is most concentrated in bovines, there are cases of Clb poisoning in humans from 2002 to 2022, with more than 4,500 cases nationwide, for example in Mexico.

EFFECTS OF CLB ON THE METABOLIC PROFILE

It is emphasized that there are very few works or almost no information on the effect of Clb on the different metabolites and enzymes at the blood serum level of bovine animals destined for human consumption. However, this study measured the various metabolites-profile. metabolic, which were detected and in which we show below:

Regarding the metabolic profile, high concentrations of transaminase enzymes were obtained: AST/GOT values in clinically healthy animals (ACS) were 533.9±0.26 U/L compared to animals treated with Clb, whose value detected was 264.7± 0.22 U/L, there was a significant decrease $p<0.01$, which indicates that there is an enormous physiological change at the liver level, at the level of the liver parenchyma, possibly degradation of the liver parenchyma without tissue destruction, since these enzymes are intracellular, The same occurred with ALT/GPT, whose value in animals not treated with Clb was 345.5±0.60

U/L compared to cattle treated with Clb, values of 277.9±0.50 U/L were detected, there is also a significant decrease in p <0.05; On the other hand, the hepatic enzyme Gamma-glutamyl transferase (γGT), significantly very low values were detected (p<0.01) with respect to the ACS animals, between 16.7±0.34 and 26.5±0.30 U/L, respectively, the Clb It is also capable of disguising certain liver pathologies, work already presented in 2011. (Figure 5), these animals presented many liver pathologies such as: cysts, obstruction of hepatic and probably renal canaliculi and although it has not been determined for this very particular case, the alkaline phosphatase-(AP).

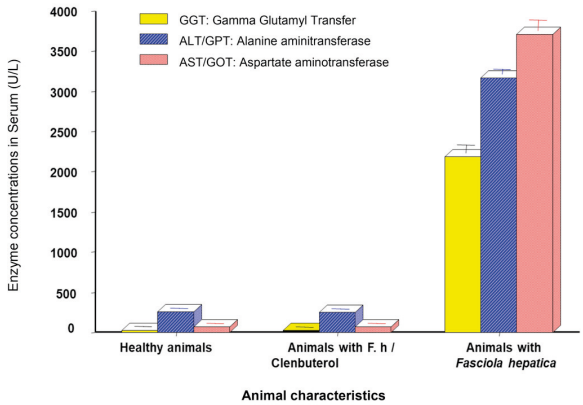

Figure 5: It is shown how Clb disguises liver diseases in cattle, where the values of animals with Fasciola hepatica plus Clb have enzymatic values very similar to clinically healthy animals, while animals with Fasciola hepatica do show liver damage - fibrosis, cirrhosis, granulomatosis and liver cancer, these due to the very high enzyme values compared to the other experimental groups.

Other metabolites such as glucose, their detected values were 115.8±0.31 mg/dL for ACS and in treated animals they had values of 195.4±0.34 mg/dL, which are low, due to the high metabolic demand that this Clb demands on the different structures, including the liver, pancreas, thyroid glands and adrenal glands, since initially these metabolic parameters are very high, as hours and days pass after ingesting this food additive this activity decreases, but certain components from the degradation of Clb accumulate, including part of the Clb ingested and that is not completely degraded, indicating that its bioavailability is useful enough to continue accumulating in tissues and continue with the degradation of lipids, with accumulation of nitrogen, through adipocytes, these as energy reserve of the body and when degraded they release fatty acids and triglycerides into the bloodstream.

The degradation of adipocytes leads to an increase in lipolysis, a decrease in lipogenesis and an increase in thermogenesis, while the muscle tends to accumulate high concentrations of Clb, which makes it anabolic for the animal with a period of prolonged action, consider that biotransformation at the muscular level, this possibly entails an increase in muscle fibers, this is due to the fact that there is an increase in protein synthesis, a decrease in protein degradation, an increase in glycolysis, in the production of lactate and oxygen utilization. In the pancreas there is a decrease in the production of insulin and an increase in glucagon, causing diabetes in the animal; while in the liver there is an increase in glycogenolysis and gluconeogenesis, this leads to liver damage in the animal such as fibrosis-cirrhosis, which was detected in 62.3% of the animals studied, as well as the presence of cysts. at the liver level.

The value detected in the prostatic acid phosphatase of the ACS animals was 3.87±0.30 U/L, compared to the animals treated with Clb, whose concentration was detected was 11.3±1.2 U/L. Most of the animals presented very high values in total, non-prostatic and prostatic acid phosphatase, this probably induced testicular thermogenesis, producing alterations in sperm morphology. All values detected with respect to the control of each of these parameters are significant: *p<0.01*. ***Figure 6.***

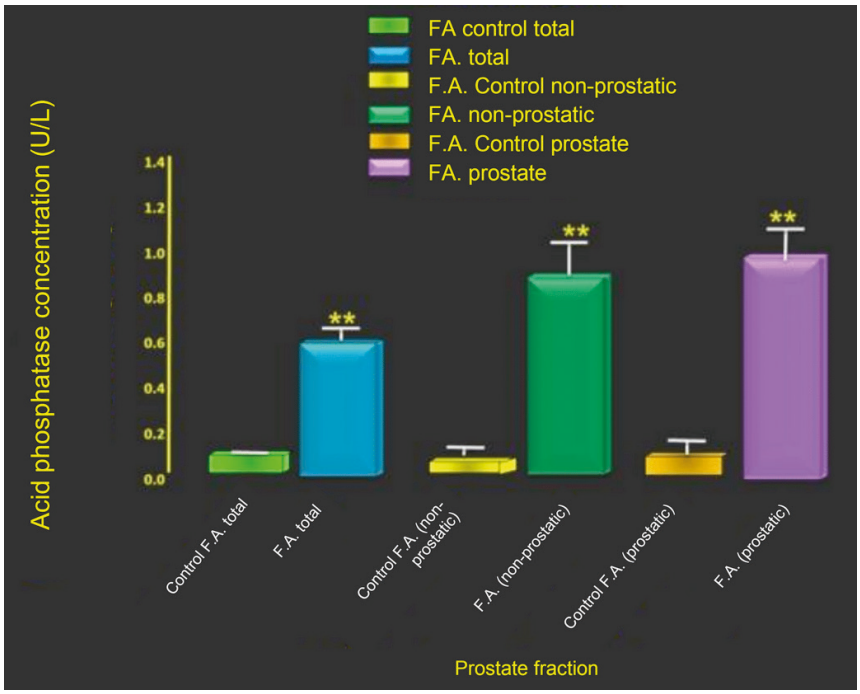

Figure 6: The values of Acid Phosphatase are shown: total, Non-prostatic and Prostatic, demonstrating that Clb produces an elevation of this prostatic enzyme, promoting alterations in sperm morphology. If the values of prostatic acid phosphatase are high as demonstrated in this study, it indicates the presence of the beginning of prostate cancer. If these values continue to be high for a very long period of time, therefore, sperm malformations were detected in almost all of them. the regions of the sperm studied.

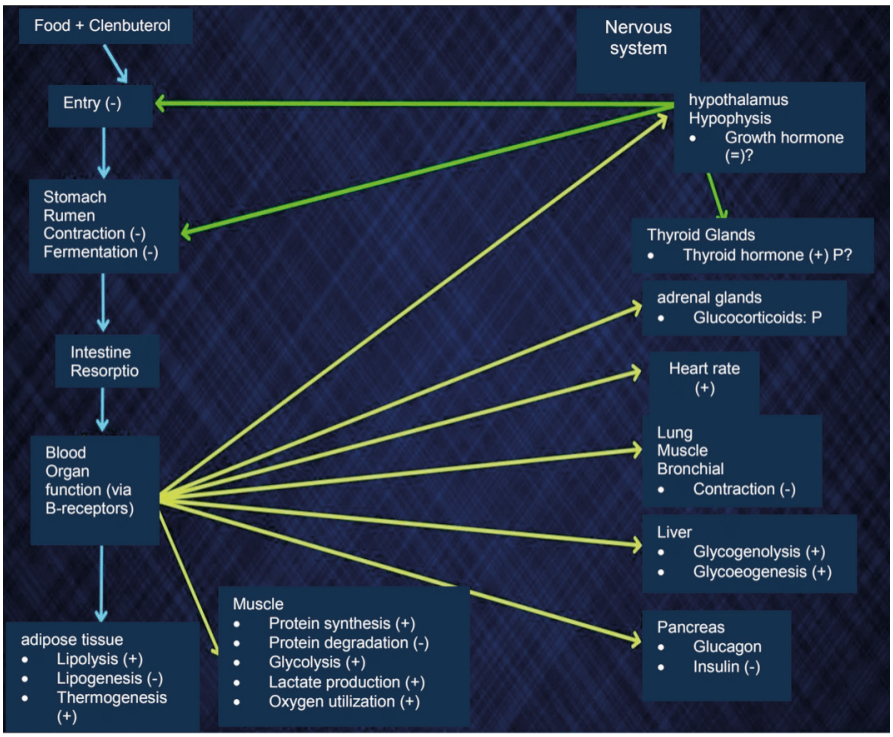

Figure 7: Effect of Clb on different organs; It will depend on the receptors that the organ has and also based on the concentration of Clb that is administered (bioavailability and biotransformation of Clb) and the age of the animal at which β2 is administered also plays a very important role. AA-Clb.

Additionally, cholesterol concentrations for this study decreased from 264.5±0.33 mg/dL to 207.8±0.48 mg/dL, this decrease is significant at $p<0.01$.

Cholesterol usually decreases when there is toroidal dysfunction induced by excess of food additives-Clb, since it is known that the thyroid gland stimulates the elimination of cholesterol by direct secretion into the bile and bile acids and also stimulates its synthesis by controlling the level of functional hepatocyte, where approximately 90% of endogenous cholesterol is formed and this can clarify the variability of changes in serum concentration in pathologies such as hyperthyroidism and hypothyroidism in cattle treated with this β2-AA-Clb, for a period of time beyond what is regulated, since many producers subject their animals to more than 100 days of treatment and to concentrations higher than the anabolic dose, between 12 and 15% higher.

As for Urea/Bun, it constitutes one of the most abundant non-protein nitrogenous components in the organism of ruminants, it is the main nitrogenous waste product of protein catabolism and is only synthesized in the liver, the values obtained for this study fluctuated between 30.4±0.23 mg/dL in ACS and in treated animals at 95.8±0.28 mg/dL. These last values greatly increased and significant ($p<0.01$). showing renal deficiency, either due to increased blood pressure at the level of the juxtaglomerular apparatus.

It is also worth mentioning that the remaining metabolites measured did not show significant changes. For this reason, we will not mention it in this study, but we do want to emphasize that all 17 metabolites measured showed changes in accordance with the ACS values. The effect of Clb on different structures of bovine animals will depend on: a) the concentration administered to the animal, 2- the time in which it is administered, 3- the age of the animal since it must be administered at an age ranging from 16 months to 24 months, which is when the animals acquire the greatest number of receptors, therefore it must also be considered that not all organs will have the same behavior to Clb and its effect may be more delayed or null since it must also be consider that not all organs have the same number of receptors. ***Figure 7.***

EFFECTS OF CLB ON STEROID HORMONES

When the values of steroid hormones linked to reproductive capacity in females were detected, estradiol (E2) values were detected between 152.1±8.91 to 1152.3±89.74 pg/ml, and progesterone (P4) values fluctuated between 248.51±12.51 at 852.42±90.2 pg/ml, these very high values of estrogen and progesterone were obtained from females with very prolonged postpartum anestrus greater than 120 days (postpartum).

It can be added to this that when the steroid hormones present high levels of estrogen it can indicate that the animal is in the estrus or heat phase, it is ready to be pregnant, however, when the levels of progesterone-P4 are elevated it can indicate that the animal is in the luteal phase which could be an indication of pregnancy, but in this study animals with both hormones were detected elevated, which may indicate that there is follicular activity but both elevated may also indicate the presence of cysts in the ovarian level of the animal producing very prolonged anestrus, this while the cysts remain, now it is also probable that this reproductive quality is due to the effect of Clb**.** ***Figure 8***

They sampled lower values of P4 and E2 hormones than females with Clb, demonstrating that Clb disguises liver conditions, since females with Clb and Fh showed a high prevalence of ovarian cysts and, at the liver level, they presented fibrosis-cirrhosis, hepatitis. and liver tumors-granulomatosis, and a high incidence of atretic follicles.

DISCUSSION

WHAT IS CLENBUTEROL HYDROCHLORIDE AND HOW DOES IT ACT IN ANIMALS OF FOOD IMPORTANCE:

Clb has a chemical structure closely related to catecholamines capable of interacting with adrenergic receptors, generally of the β2 type. Chemically it is an amino-4-amino alpha T butylamino methyl 3,5 dichlorobenzyl alcohol (Figure 2). Its half-life is long-acting, with the particularity of being able to be stored in the liver and kidney. According to Boato, (2000); Dimaano, (2008) and Valladadares et al., (2013b), it is metabolized through N-oxidation reactions into hydroxyclenbuterol and glucuronic conjugates.

The Clb considered a synthetic food additive and belonging to a class of medications physiologically analogous to adrenaline (Mersmann, 1998). Chemically it is described as a white, anhydrous powder, very soluble in water and highly stable at room temperature, its melting point is 174 to 175.5 ºC, it has the ability to interact with adrenergic receptors, generally of the β2 type (β2-agonist), (Ishikawa, 2009; Valladares et al., 2013 a and 2014a, b and c). Physiological adrenergic agonists (β-adrenergic) are norepinephrine and epinephrine; Norepinephrine is a catecholamine from the phenethanolamine group and is also considered a neurotransmitter of the sympathetic nervous system, which is biosynthesized from tyrosine and circulates in the blood serum in relatively high concentrations. Adrenaline from the same group is synthesized and secreted in the adrenal medulla; It circulates in lower concentrations than norepinephrine in most mammals, but in situations of stress it responds in a greater proportion than norepinephrine. Norepinephrine is more selective for α receptors and adrenaline acts on both, with greater selectivity for β receptors, but with a more dominant α effect.

Physiological responses occur when these β2-adrenergic agonists bind to specific receptors (Badino *et al*., 2005).

These are organic molecules and β2-adrenergics that bind to β2-adrenergic receptors, giving rise to the agonist-receptor complex (β2AR), which in turn activates the Gs protein. The ά subunit of the Gs protein activates adenylate cyclase (ADC-ase), an enzyme that produces cyclic adenosine monophosphate.

(cAMP) is considered one of the main intracellular signaling molecules. This molecule produces its effects by binding to the regulatory subunit of protein kinase A, to release the catalytic subunit that phosphorylates a good number of intracellular proteins (Mazzanti et al., 2003). These proteins have vital functional roles for a varied range of functions that will allow the entry of Ca++ into the cell, to mediate the synthesis of key proteins for cellular functioning (Mazzanti et al., 2003).

Clb is administered at doses between and ten times higher than the therapeutic one, at these concentrations it must have an anabolic action, this will favor protein synthesis and reduce fat in the skeletal muscles of the bovine. βAR receptors are present in most mammalian cells, although the distribution of the subtypes (β1, β2 and β3) and the proportion of each of these varies depending on the tissues and species of animal (Mitchell and Glora, 1998).

The β1 is considered to predominate in the heart, stimulating its inotropism (force of contraction) and in the intestinal smooth muscle, inducing relaxation, and the β2AR are located in the bronchi and uterine muscle, causing relaxation in both cases, therefore, the magnitude of The physio-pharmacological activity of a β-adrenergic agonist or partial agonist will depend on its so-called intrinsic

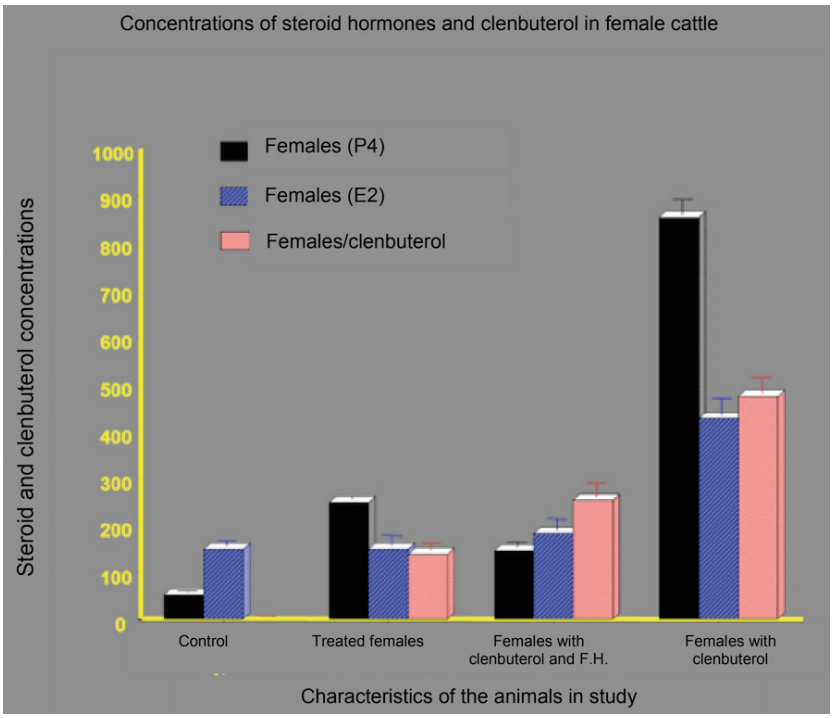

Figure 8: The concentrations of steroid hormones in female cattle are shown:

a) control animals, b) females treated with Clb, c) females with Clb and Fasciola hepatica-Fh and d) females with clenbuterol, it can be seen that the values of females with Clb and Fh

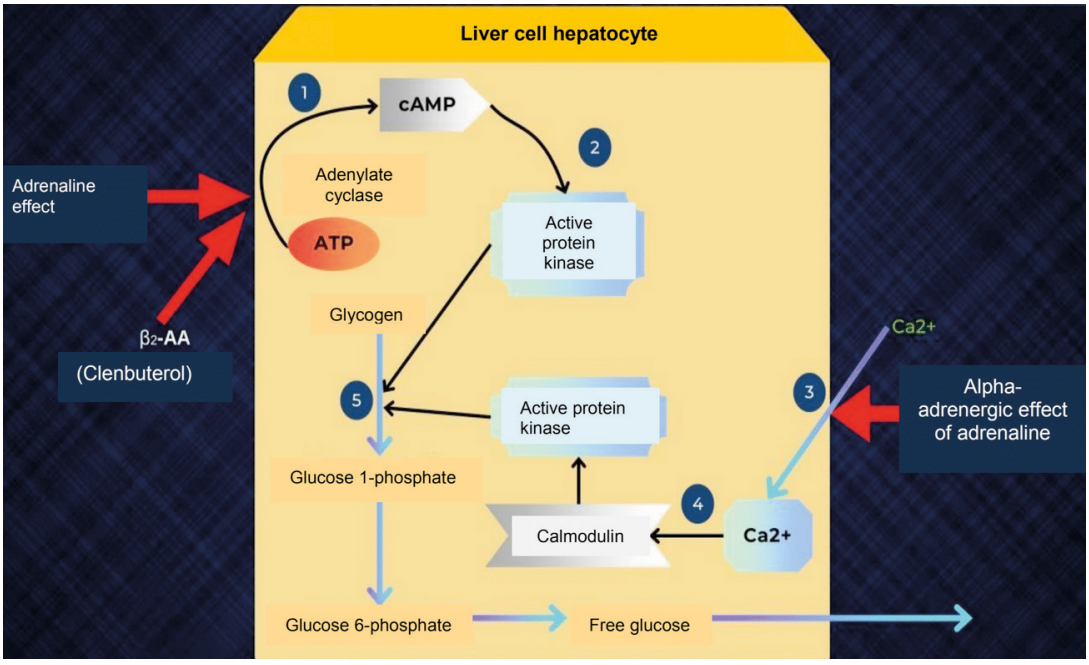

Figure 9: It details how adrenaline acts on liver cells - hepatocytes and β2AA, using the same receptors and the same cytoplasmic enzymes to finally produce glucose at the liver level. From glycogen, which is stored as an energy reservoir in the liver.

activity in the receptor and distribution in the target tissues (Valladares et al., 2014 a, b).

Regarding Clb, its effect in physiological conditions produces muscle growth skeletal, which is the primary result of hypertrophy and is detected with an increase in muscle protein synthesis and a decrease in muscle protein degradation or a combination of both produces an increase in muscle mass (Mersmann, 1998). This allows increasing blood flow to certain regions of the body, which allows the process of hypertrophy in the skeletal muscle by transporting greater amounts of substrates (a.a and proteins) and energy sources for protein synthesis (Ramos, 2009). Another of its main actions at the muscular level is the decrease in the amount of fat in the carcass. The degradation of triacylglycerols in adipocytes and the inhibition of the synthesis of FA-fatty acids and triacylglycerol have been demonstrated "in vitro", the animal tissue presents increased lipolytic activity (decrease in muscle fat), decreased hypogenic activity, or both (Valladares et al., 2013 a and c). The elevation of the plasma concentration of non-esterified fatty acids after the administration of Clb confirms the lipolytic activity that occurs in adipocytes (Caicedo et al., 2011 and 2021).

Clb increases blood perfusion to the muscle, as well as greater availability of energy and amino acids; As a result, there is an increase in protein synthesis and retention, which will favor muscle hypertrophy, mainly of the muscles of the animal's hindquarters. In muscle, in addition to hypertrophy, changes occur in the type of muscle fiber, there are also changes in the proportion of transcription RNA for muscle proteins such as myosin and actin (Meyer and Rinke, 1991). In sheep and cattle, it has been observed that muscle weight increases by 40%, and that the magnitude of the response varies depending on the β-adrenergic supplied, as well as the influence of factors such as species, breed, age, sex and diet (Barry and Graham, 2013 and Caicedo et al., 2021).

According to Mersmann, (1998) and Caicedo et al., (2021), Clb is considered a Beta2-adrenergic agonist (β2-AA) and a non-steroidal anabolic currently used for doping in sports. This substance is known to induce hypertrophy of musculoskeletal tissue. The precise mechanism of how this hypertrophy effect is produced is not very well elucidated; However, the most accepted hypothesis is that it induces this effect through β2-adrenergic receptors, by controlled regulation of the expression of insulin growth factors (IGFs) that play an essential role in development, growth and regeneration of musculoskeletal tissue.

Histologically, skeletal muscle satellite cells are mononucleated and reside between the sarcolemma and basal lamina of adult myofibrils. In response to stimuli such as mechanical loading, unloading, denervation, and injury, they are activated through several growth factors that contain IGFs (Boato, 2000; Mersmann, 1998). This activation allows for adaptive changes such as hypertrophy., the alteration of the type of fibers and regeneration (Valladares et al., 2014b).

For β2AA, the replacement of the aromatic ring is very important; its replacement allows a specific biological activity at the muscular level, since it is done to obtain a defined biological activity. It must be considered that when the OH (hydroxyl) are replaced by a halogen, as in the case of Clb by chlorine, biotransformation by COMT enzymes (catechol-O-methyltransferases) is avoided at the tissue level and the hepatic biotransformation (Boato, 2000), the presence of this chlorine halogen makes it more fat-soluble than its analogues, and, therefore, it tends to diffuse more in tissues and animal fat (Mersmann, 1998; Valladares et al., 2014b, Caicedo et al.,2021).

EFFECT OF CLB DETECTED IN THIS STUDY

For this study, we must consider that one of the most important characteristics regarding the structure of Clb is that it has a halogen in its chemical structure, which is the chlorine ion. This chlorine ion (Cl) allows the metabolic activity of Clb to be delayed., therefore, its effect is much longer than other β2-AA agonists and its total excretion is also more delayed (Martin 1971, Ruffolo, 1991, Waldeck and Widmark, 1995). Furthermore, the effect that Clb has on the activities of the reproductive system in females and males is not very well elucidated, because in animals with a high intake of Clb, reproductive activity decreases (Caicedo et al., 2010 and 2011, Paz-Calderón et al., 2011), on the other hand, there is an increase in prostatic acid phosphatase possibly due to the thermogenic effect of Clb; what this caloric phenomenon does is alter the morphology of the sperm (Paz-Calderón et al., 2011). Furthermore, we can consider that β2-AA stimulate the adrenal gland by producing glucocorticoids and corticosteroids (dexamethasone and betamethasone); The effect of Clb on this adrenal gland is still not clear. It is likely that in females it stimulates production of steroids (P4 and E2) in the ovaries such as progesterone and estradiol and in males increases the levels of testosterone (T) and prostatic acid phosphatase, however, the probable damage that clenbuterol can cause in the reproductive organs is unknown. (Caicedo et al., 2011; 2021 and 2023), because these steroids are produced there, it is likely that Clb in the adrenal glands increases glucocorticoids and mineralocorticoids, which can also increase steroids (estrogens and progestins) through this organ (Saavedra et al., 2019, Caicedo et al., 2011, 2021 and 2023). It can be considered that Clb produces an increase in the activity of the nervous system, which leads to loss of appetite, which may be due to the animal's feeling of discomfort or glycogenolytic and lipolytic activity, blocking the appetite centers through overload signals from chemostatic receptors (Caicedo et al., 2009 and Saavedra et al., 2019). Being able to cross the blood-brain barrier, it is feasible that the reduction of food consumption can be attributed to excess stimulation of β2-adrenergic receptors (β2-AR) at the level of the central nervous system.

The growth-promoting effects exerted by Clb are strongly mediated by the direct stimulation of β2-adrenergic receptors (β2-AR) (Helferich et al., 1990, Ni et al., 2010), located in muscle tissue and also, indirectly due to variations in plasma concentrations of catabolic hormones or anabolic (Higgins et al., 1988), such as glucocorticoids, growth hormone (GH) or insulin. If hormones can alter the response of adipose tissue to endogenous catecholamines, they can also affect the response of skeletal muscles to exogenous β2 agonists (Sumano et al., 2002). The study verifies that Clb, therefore, modifies the muscle composition of the animals, since in animals treated with β2-AA, an increase in protein deposition (15%) and a decrease in fat deposits is observed (18%) (Lueso and Gómez, 1990).

Muscle growth, in response to treatment with β2-AA, is a hypertrophy of striated skeletal muscle tissue, which is demonstrated by the studies carried out by Beermann et al., (1983 and 1986) in rats and by Martin et al., (1990) in cows. The effects of β2-AA on the endocrine system are largely due to the release of other hormones (Caicedo et al., 2009, 2011; Saavedra et al., 2019 and Caicedo et al., 2023).

Among the actions of catecholamines are the inhibition of insulin secretion, the increase of glucagon and the stimulation of the release of adrenocorticotropic hormone (ACTH), somatototropic hormone (STH) and gonadotropins such as: FSH and LH,

(Beermann et al., 1987). However, there is a surprising lack of information on the effects of Clb on the adrenal gland, which is even more surprising, if you consider that there are β-adrenergic receptors (β-AR) in this gland, that the adrenal medulla is one of the tissues that synthesize and secrete the natural catecholamines adrenaline and norepinephrine and that the adrenal gland synthesizes and secretes glucocorticoids, finally, it must be considered that the direct involvement of this gland in the body's adaptation mechanisms to stress, both in the short and long term.

The effects of β2-AA on fat metabolism are very difficult to define, however, they act indirectly on fat deposition, by increasing the metabolic speed and energy expenditure of treated animals and by reflecting thermogenesis, a part of the energy ingested prevents the formation of fat and on the other hand the direct effect is in the increase in the levels of cyclic AMP in the adipose tissue, ATP is transformed into cyclic AMP that activates certain proteins such as protein kinases that by Phosphorylation stimulates an intracellular lipase that transforms triglycerides into fatty acids and glycerol. This mechanism increases lipolysis and decreases lipogenesis. Likewise, this mechanism will depend on the animal species in which we are treating, which is why the increase in the administered concentration and the time in which the animals are subjected to this β2-AA plays a role. a very important role in the short and medium term effect, for its administration. Based on previous studies and the data obtained in this study, the following can be added: The administration of Clb, at an anabolic dose, causes an alteration in the functionality of the hypothalamic-pituitary-adrenal-gonadal-hepatic axis in bovines., which in some cases is reversible, after a period of withdrawal or quarantine. In addition, the metabolic pathways of Clb in cattle, which entails the formation of: 4-nitro-clenbuterol, hydroxylamine of clenbuterol, 4-amino sulfonic acid of clenbuterol, alcohol-hydroxylamine 2,5-dichloro-a-benzyl and acid 4-amino-3,5-dichloromandelic, all these components are highly toxic for animal physiology according to Fiems (1987), Zalko 1997 a and b; Caicedo et al., (2021). Figure 10.

The maximal response of β2-AA-clenbuterol can be considered to be affected by the dose administered and the duration of a sustained dose (Ricks et al., 1984). This has been well documented and that the β-AR-mediated increase in cAMP is temporary and that continued activation of the receptor by the β-AA ligand is necessary to maintain cAMP levels to sustain the response (Figure 7). Exposure to a constant dose of βAA to the recipient will eventually cause an acute desensitization or inactivation of receptor-mediated signaling.

Phosphorylation of both protein kinase A-(PKA) and β-AR kinases are the main steps in initiating the signaling cascade following cAMP activation by β-AR (Hausdorff et al., 1990). Acute desensitization can be avoided to some extent by increasing the dose and enhancing the signal.

The long-term (chronic) exposure to high doses of β2-AA leads to internalization or loss of the cell surface receptor and decreased abundance of β-AR mRNA (Hausdorff et al., 1990). These alterations appear to be irreversible at least in the short-term feeding period. However, in addition to all this, Sauer et al., (1995) detected that when evaluating the kinetics of Clb in Holstein-Friesian calves, to which a dose of 10 μg/kg of weight was given every 12 h for 21 days, found residues in blood serum at 6 h, 1, 2, 4, 8 and 16 days after starting the treatment. From day 2 of Clb withdrawal, to the slaughter of the cattle, the highest concentrations detected were found in the liver, kidney, bile and urine.

In studies carried out in Rattus novergicus, according to FAO-WHO, they considered that the administration of Clb increased the incidence of meso-ovarian leiomyomas after receiving doses of up to 25 mg/kg of weight per day. They concluded that these are due more to adrenergic stimulation and not so much to genotoxic factors, the same can be said that happened with mice exposed to Clb, according to Valladares-Carranza et al., (2017), where there was an increase in muscle mass and alterations at the level of the liver and heart in this were detected through the histopathological study of the cardiac fibers, thickening and curling of muscle fibers, pleomorphism and nuclear spinning, this related to muscular hypertrophy of the cardiac muscle. While at the liver level of the animals treated with Clb the lesions observed were: swelling and moderate to diffuse hydropic degeneration, mitosis, pyknosis and megalocytosis with megakaryosis of hepatocytes, this causes lesions through the hepatocytes causing alterations in their production. of bile, hence the high concentrations of bilirubin in cattle detected in this study. In the liver of mice exposed to Clb, important alterations were observed, which reveal its toxic effect (Valladares-Carranza et al., 2014a and b and 2015 and Caicedo et al., 2023). Clb in the organism of animals has a prolonged function and action, as mentioned it tends to be stored primarily in the liver and kidney (as these organs are filtering components and regulators of homeostasis) and is metabolized through reactions of N-oxidation in hydroxyclenbuterol and glucuronic conjugates (Mazzanti et al., 2003 and Valladares-Carranza et al., 2015). Zalko et al., (1997a), stated that the metabolites of Clb: N-hydroxylarylamine and N-nitroso clenbuterol, have toxic properties that pose a risk to both animal and human health.

Another very particular case of the effect of Clb in rabbits to which doses of 30 μg-50 mg/kg body weight per day were administered, signs of fetus toxicity were observed, such as delayed ossification and cleft palate. On the other hand, studies carried out by Carrola et al., (2003), in two human cases they presented signs of muscle tremor, nausea and incoordination; On auscultation they showed an increase in heart rate, an increase in blood pressure and in the hematological examination they presented: leukocytosis, accompanied by neutrophilia, hyperkalemia and hyperglycemia, which occur at the level of bovines that are subjected to feeding with β2AA-Clb. It would be very interesting to carry out research of this type in cattle, poultry or pigs, in addition to the fact that they are the species in which it is used the most, and those that are consumed in greater frequency and quantity in the national market (SIAP, 2009). According to studies by Avilés-Martínez et al., (2019), Clb values were detected in high concentrations in cattle in 69.3% of the animals sampled in municipal slaughterhouses, other studies by Caicedo et al., (2021) detected 62.3% of animals. positive to Clb from a population of 36,000 cattle sampled in municipal slaughterhouses, indicating that there are no relevant measures for the use of this component (Caicedo et al., 2021 and 2023), despite the fact that the prohibition rule exists in Mexico (Official Standard Mexican NOM061-ZOO-1999, which prohibited its use, production, marketing and administration in animals for human consumption in the country (SAGARPA, 2000) and that since 2002 poisonings in humans have been occurring due to it.

Finally, β2-adrenergic agonists are analogues of the catecholamines epinephrine and norepinephrine. They may function through specific beta-adrenoceptors (βAR)

on the surface of adipocytes and skeletal muscle cells. It has been proven that β2AA. Clenbuterol promotes growth, and it has been elucidated to some extent that these drugs Clb and zilpaterol reduce total carcass fat and increase total carcass protein in four species: cattle, sheep, domestic poultry and rats. Many β-adrenergic agonists reduce carcass lipids by stimulating lipolysis and blocking lipogenesis in adipose tissue.

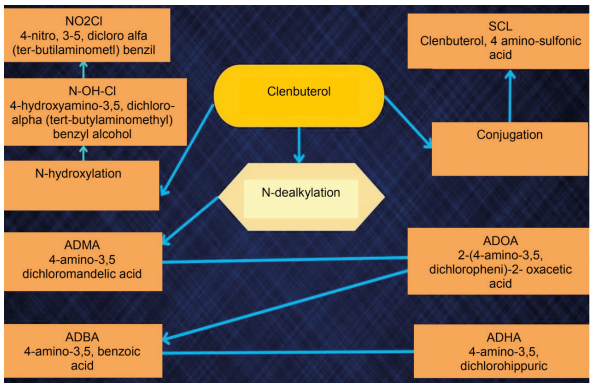

Figure 10: Bioavailability and Biotransformation of Clenbuterol in cattle and possible metabolic routes of Clenbuterol in the different structures of cattle: liver mainly, pancreas, kidney, and lung at the level of the nervous system and adrenal glands, according to Fiems (1987), Zalko 1997 a and b and Caicedo et al., 2011 and 2021.

CONCLUSIONS

For this study we can consider the following conclusions:

1- Clb contributes to increasing muscle mass in cattle at the expense of altering their well-being, causing alterations in blood metabolites linked to homeostatic processes, "physiological imbalance"

2- Therefore, it affects the genes that regulate cellular and organ metabolism involved in this process, which are linked to the increase in the animal's muscle mass.

3- The accelerated hypertrophy produced by this β2AA-Clb

4- it is not ideal due to the time in which it occurs (15 to 60 days), altering hormonal processes and the central and peripheral nervous system,

5- Clb contributes to alterations in the production of steroid hormones, altering ovarian activity in females, producing very prolonged anestrus; it also usually inhibits milk production.

6- An excess in the dose of Clb produces alterations in the levels of prostatic acid phosphatase, possibly causing prostate cancer in cattle, also producing testicular atrophy.

7- While in males it produces alterations at the level of the precursor cells of spermatogenesis, the spermatogonia, it produces malformations at the tail and head of the sperm,

8- The organ that produces the greatest alterations is the liver, causing cysts, liver fibrosis, telangiectasias, altering the function of hepatocytes in producing bile and accumulating this in the bile canaliculi, producing alterations at the structural level of the liver, possible Granulomatosis hepatica.

9- Clb masks many liver pathologies such as hepatitis, cirrhosis, granulomatosis hepatica, which is not detected with the naked eye, but with biopsy studies and also makes the detection of fascioliasis in ruminants difficult.

10- In Mexico, the use of Clb is not yet considered a public health problem, despite the fact that the laws prohibit the production, distribution, marketing and finally the administration for the fattening of animals of food importance.

However:

a) The β_2-AA- Clenbuterol consistently improves beef cattle performance and increases muscle growth when mixed with finishing rations.

b) Changes in mRNA abundance of multiple genes associated with myogenic differentiation may indicate an important effect of β_2AA on the proliferation, differentiation and/or recruitment of satellite cells into muscle fibers to promote muscle hypertrophy

c) The physiological activity of Clb depends on the inherent activity of the receptor and its absorption, rate of metabolism, elimination and distribution in the target tissue.

d) It must be considered that cattle that receive β2-AA-clenbuterol tend to have very low marbling scores, less back fat and greater meat hardness and high levels of H2O content, around 30%. more than an organically fed animal.

e) Elevation of glycolytic fiber types with treatment with β2-AA-clenbuterol is mainly responsible for the increase in muscle hypertrophy, however, it is negatively correlated with the amount of adipose tissue, both intramuscular and intermuscular,

f) Finally, the availability and biotransformation of Clb in tissues and organs will depend on the concentration and time in which the cattle are subjected to this anabolic, which, there is evidence that they are administered more than the allowed anabolic doses, and for a longer time stipulated in the guidelines for the administration of synthetic anabolics in domestic animals of food importance, which must not be, since it affects the animal itself, its well-being and finally the consumer, man.

Although there is a prohibition (regulations: Standard 069-1999) in its application to animals of food importance, this administration is not regulated by professional personnel.

THANKS

To the research authorities of ``*Benemérita Universidad Autónoma de Puebla*``, to the municipal slaughterhouses that participated in authorizing the taking of blood samples and organs from the bovines and private farms involved, as well as to all the students who participated in this research study. everyone, thank you.

We clarify that this work does not have any type of particular interest, it only wants to demonstrate that it is necessary to develop a lot of research in order to increase the sustainable quality of the animal's life and its well-being, to ensure the production of safe food in our region and improve the environment.

Likewise, it must be clarified that the doses of Clb administered were not given by the authors of this study, but that the farmers were already administering doses from 5 to more than 10 times its therapeutic concentration, and that the authors carried out supervision over the administration of this β2AA, in farms where we were authorized, since in many we were not allowed to enter. It must be noted that many samples were carried out on private farms and mostly in municipal slaughterhouses, where this additive is already a problem, therefore, we do not have any type of responsibility or influence in the administration of this component β2AA-Clb, and the animals Before sampling, they underwent veterinary inspection by the pathologist of each slaughterhouse. Our function was only blood sampling, palpation of livers, lymph nodes and obtaining organs, mainly from those with visible pathological signs.

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