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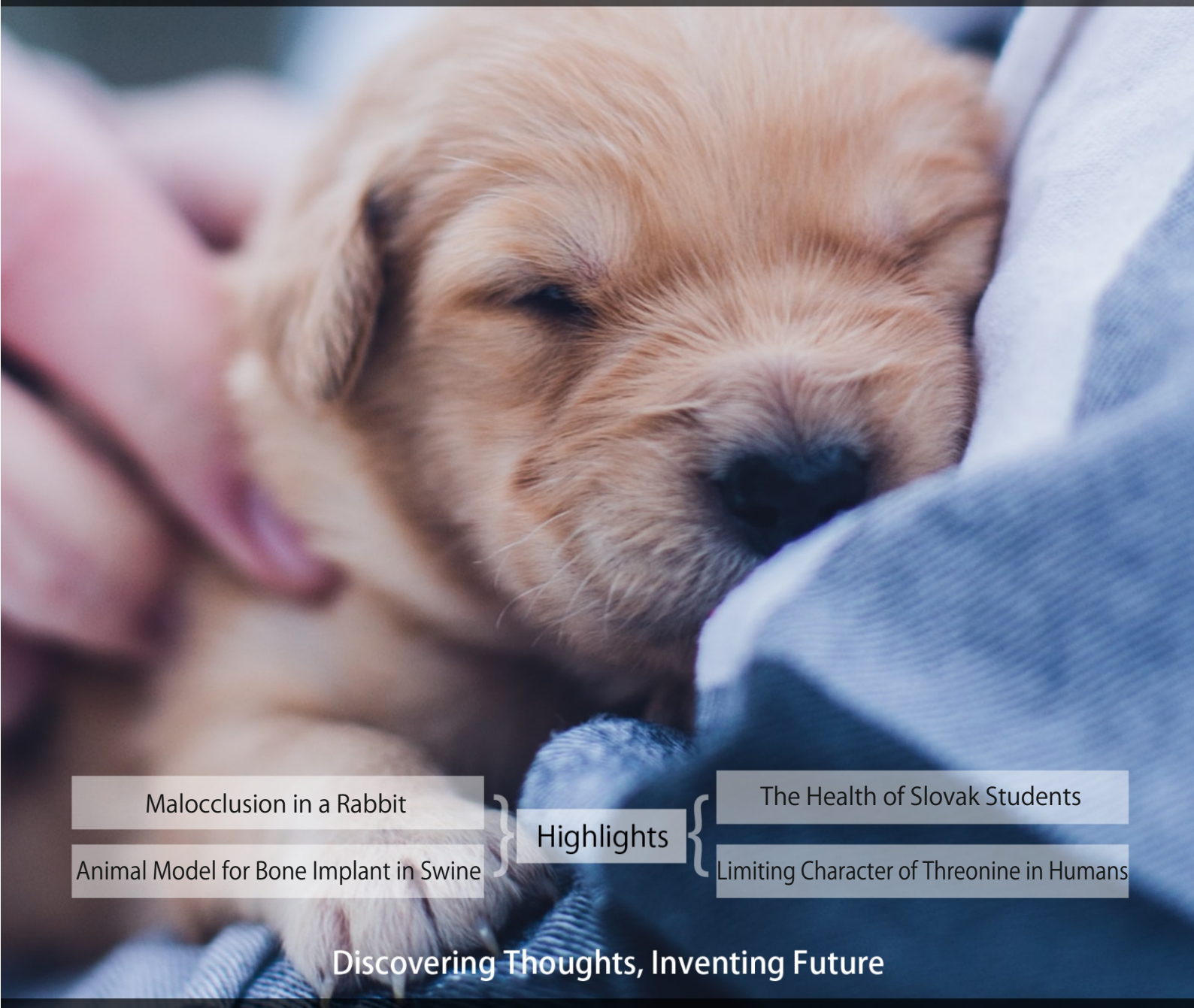

Malocclusion in a Rabbit

Animal Model for Bone Implant in Swine

Highlights

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Limiting Character of Threonine in Humans

Discovering Thoughts, Inventing Future

VOLUME 23 ISSUE 1 VERSION 1.0

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Biochemical Reasons for the Presence of the Limiting Character of Thrheonine in Some Mammals and the Absence of the Limiting Character of Threonine in Humans

By A.V. Malinovsky

Abstract- The same essential amino acids can be limiting for one kind of animals and cannot be limiting for the others which have the same ration. In particular, threonine is a limiting amino acid for pigs that are fed with grains but it is not limiting for human. As for lysine it is a limiting amino acid for all kinds of animals.

In the present article the reasons for limiting character of threonine and the absence of this character with reference to pigs and human are considered on the biochemical level. Besides here the reasons for limiting character of one or another essential amino acid are analysed, which is very important for making up ration.

Keywords: threonine, transamination, pigs, a human.

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Biochemical Reasons for the Presence of the Limiting Character of Thrheonine in Some Mammals and the Absence of the Limiting Character of Threonine in Humans

БИОХИМИЧЕСКИЕ ПРИЧИНЫ НАЛИЧИЯ ЛИМИТИРУЮЩЕГО ХАРАКТЕРА ТРЕОНИНА У НЕКОТОРЫХ МЛЕКОПИТАЮЩИХ И ОТСУТСТВИЯ ЛИМИТИРУЮЩЕГО ХАРАКТЕРА ТРЕОНИНА У ЧЕЛОВЕКА

A.V. Malinovsky

Абстрактный- **Одни и те же незаменимые аминокислоты могут быть лимитирующими для одних видов животных и не быть лимитирующими для других при сходном рационе. Так, треонин является лимитирующей аминокислотой при зерновом питании для свиней, но не является лимитирующей для человека. При этом лизин является лимитирующей аминокислотой для всех видов животных. В статье на биохимическом уровне показаны причины наличия лимитирующего характера треонина и отсутствие лимитирующего характера треонина на примере свиньи и человека, а также анализируются причины лимитирующего характера той или иной незаменимой аминокислоты, что представляет важность для составления рационов.**

КЛЮЧЕВЫЕ СЛОВА: треонин, пераминирование, свиньи, человек.

Abstract- The same essential amino acids can be limiting for one kind of animals and cannot be limiting for the others which have the same ration. In particular, threonine is a limiting amino acid for pigs that are fed with grains but it is not limiting for human. As for lysine it is a limiting amino acid for all kinds of animals.

In the present article the reasons for limiting character of threonine and the absence of this character with reference to pigs and human are considered on the biochemical level. Besides here the reasons for limiting character of one or another essential amino acid are analysed, which is very important for making up ration.

Keywords: threonine, transamination, pigs, a human.

Введение

Известно, что белок синтезируется по принципу «все-или ничего», т.е. при отсутствии в пуле хотя бы одной аминокислоты молекула белка не синтезируется. Такая отсутствующая аминокислота называется лимитирующей Необходимо отметить, что в здоровом животном организме лимитирующей может быть только незаменимая аминокислота, т.к. заменимая в подобном случае синтезируется и доставляется в рибосому, где собирается молекула белка. При этом видовые различия животных могут влиять на лимитирующий характер той или иной незаменимой аминокислоты. Так, House et al. [1] заявляют, что треонин- обычно вторая или третья лимитирующая аминокислота в питании свиней. основанном на зерновой и соевой пище. Однако более полувека назад Институт питания АМН СССР под руководством академика А.А. Покровского установил, что для человека из 8 незаменимых аминокислот лимитирующими являются лизин, метионин и триптофан, причем в первую очередь это касается рациона, основанного на зерновом питании. Для объяснения этого факта необходимо проследить разницу в превращении треонина у свиньи и у человека.

I. Особенности Превращения Треонина у Млекопитающих

Особенности катаболизма треонина у млекопитающих. Chapman K. [2] отмечает, что у млекопитающих имеется два пути катаболизма треонина: он может расщепляться треониндегидратазой в цитозоле до NH_4^+ и α-кетомасляной кислоты, которая быстро и необратимо распадается до CO_2; а также он может метаболизироваться треониндегидрогеназой в митохондриях до α-аминоацетоуксусной кислоты, которая затем обратимо расщепляется аминоацетонсинтетазой до глицина и ацетил-КоА. Последнее маловероятно из-за крайней неустойчивости α-аминоацетоуксусной кислоты, которая прежде, чем подвергнуться действию какого-либо фермента, самопроизвольно декарбоксилируется в аминоацетон, который окисляется в аминоацетоновом цикле до конечных продуктов.

В работе Moundras et al. [3] показано, что в гепатоцитах крысы 65 % окисления треонина

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осуществляется глициннезависимым треониндегидратазным путем — распад под действием треониндегидратазы. Позднее House et al. [1] это подтвердили своими исследованиями также с гепатоцитами крысы.

Поскольку треонин является незаменимой аминокислотой, оба фермента должны расщеплять его углеродный скелет необратимо. Если необратимость действия треониндегидратазы не может вызывать сомнений, то необратимость действия треониндегидрогеназы нуждалась до недавнего времени в специальных доказательствах, т.к. дегидрогеназы, как правило, действуют обратимо. В последние годы на основании работ [4-7] доказана необратимость действия треониндегидрогеназы у млекопитающих [8-11]. Причем в работе [5-7] в качестве объекта для изучения треониндегидрогеназы использовались крысы. Таким образом, опыты с крысами ярко демонстрируют невозможность синтеза у млекопитающих углеродного скелета треонина, иными словами, что треонин-незаменимая аминокислота.

Возможность переаминирования треонина у млекопитающих. Как известно, единственным путем биосинтеза незаменимых аминокислот, кроме метионина, у животных является обратимое переаминирование кетоаналогов этих аминокислот с некоторыми аминокислотами; метионин может также подвергаться обратимому (в итоге) переметилированию, рассмотрение которого выходит за рамки статьи.

Elliott и Neuberger [12] провели два эксперимента. Первый эксперимент был проведен на кроликах с относительно малым количеством глицина, содержащего ^{15}N. Второй эксперимент был проведен на крысах. В нем количество ^{15}N-глицина было почти в 4 раза выше, чем в эксперименте на кроликах. Включение ^{15}N при этом было значительно выше как в белках внутренних органов, так и во многих отдельных аминокислотах, особенно в глицине и образующемся из него серине, в то время как лизин и треонин не показывали повышенного содержания ^{15}N. Отсюда Elliott и Neuberger [12] сделали вывод, что треонин, подобно лизину, не принимает участия в обратимом переносе азота, который наблюдается у других аминокислот, как заменимых, так и незаменимых. Данный стереотип сложился в традиционной биохимии. Так, в [1] говорится, что клетки млекопитающих не обладают необходимыми ферментами для переаминирования треонина и потому поступление этой незаменимой аминокислоты в катаболические пути представляет ее необратимую потерю для синтеза белка. В этой работе также сообщается, что треонин-вторая или третья лимитирующая аминокислота в питании свиней, основанном на зерновой и соевой пище, как сообщалось выше. При этом ничего не говорится о лимитирующем характере треонина для крыс, на которых была выполнена данная работа.

Между тем Meltzer и Sprinson [13] показали, что после кормления крыс ^{15}N-меченным лейцином очень малое количество ^{15}N было обнаружено в треонине, составляя только 2% такового, найденного в глутаминовой кислоте, и менее 1%, выделенного в лейцине из тех же органов. В книге Майстера [4] на основании работ [12,13] делается вывод, что раз некоторое количество азота лейцина все же было обнаружено в молекуле треонина, в организме животного происходит незначительный синтез треонина или же реакции его расщепления частично обратимы. Майстер также не исключает, что отмеченное включение изотопного азота в треонин, возможно, обусловлено действием микрофлоры кишечника. Но если бы последнее имело место, то столь же малое количество азота лейцина было бы обнаружено и в лизине, а этого не наблюдалось [13]. Майстер исключает образование треонина из гомосерина в тканях животных. Невозможность синтеза треонина из гомосерина у животных в XXI веке подтверждена Donini et al. [15].

Т.Т. Березов в своей монографии в 1969 г. указал как на доказанный факт, что в тканях млекопитающих механизм переаминирования является главным путем дезаминирования L-аминокислот и перечисляет все аминокислоты, участвующие в этом процессе [16]. Среди аминокислот Т.Т. Березов называет треонин, но не называет лизин, следовательно, он имеет в виду сами аминокислоты, а не продукты их превращения, потому что продукт превращения лизина — α-аминоадипиновая кислота — активно подвергается переаминированию.. Т.Т. Березов в своей работе [16] также отмечает, что переаминирование может происходить в тканях между разнообразными монокарбоновыми донорами и акцепторами аминогрупп без участия дикарбоновых аминокислот. К реакциям этого типа относятся процессы переаминирования между рядом аминокислот и пировиноградной кислотой с образованием аланина и соответствующих α-кетокислот, протекающие в митохондриях печени. Была показана и обратимость этих реакций, а также различная способность отдельных тканей катализировать описанные превращения. Дальнейшие исследования подтверждают возможность переаминирования треонина у крыс. Так, в работе Noguchi T. et al. [17] рассматривается фермент серин-пируватаминотрансфераза, выделенный из митохондрий печени крыс и катализирующий переаминирование различных аминокислот как с пировиноградной, так и с фенилпировинградной кислотой. Причем если фенилаланин весьма активно переаминируется серин-пируватаминотрансферазой с пировиноградной кислотой, то лейцин (в большой степени), треонин (в меньшей степени) и глицин (в очень малой степени) переаминируются ею только с фенилпировиноградной кислотой (схема 1).

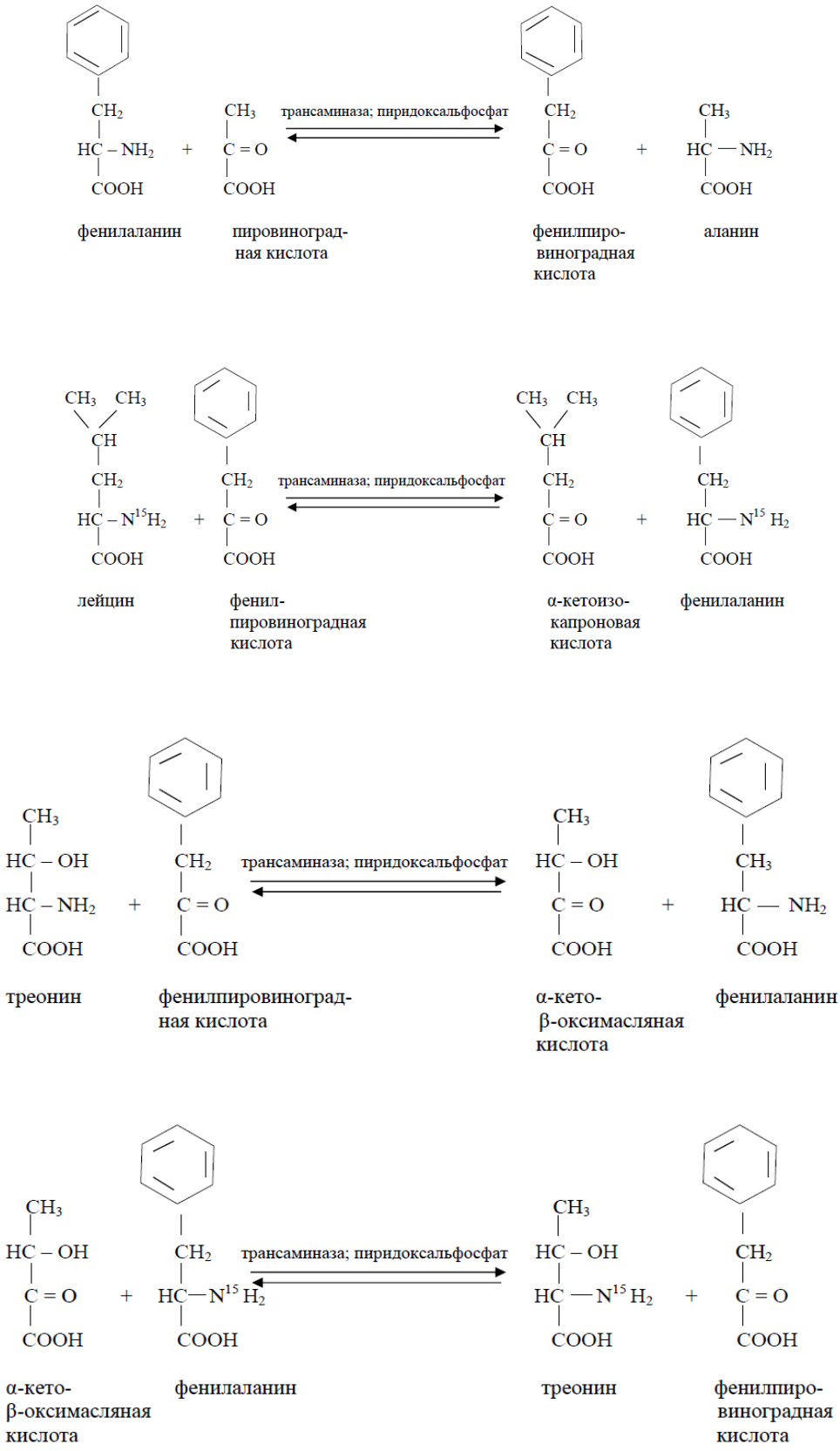

Схема 1: Обратимое переаминирование лейцина и треонина с фенилпировиноградной кислотой.

В работе Ishikawa et al. [18] устанавливается идентичность кинуренинаминотрансфераз почек и мозга крыс. Что касается субстратной специфичности обоих ферментов, то кроме кинуренина (продукт распада триптофана), они способны катализировать переаминирование многих аминокислот как с пировиноградной, так и с фенилпировиноградной кислотами. Однако если кинуренинаминотрансфераза мозга хорошо переаминирует почти все аминокислоты с фенилпировиноградной кислотой, то кинуренинаминотрансфераза почек не переаминирует с ней некоторые аминокислоты. Треонин переаминируется с фенилпировиноградной кислотой обеими кинуренинаминотрансферазами, но в почках в небольшой степени, а в мозге так же активно, как и другие аминокислоты, уступая лишь метионину. С пировиноградной кислотой обе кинуренинаминотрансферазы переаминируют только ароматические аминокислоты. На лизин обе кинуренинаминотрансферазы не действуют, что подтверждает отсутствие у него способности к переаминированию. Следовательно, катализируемое кинуренинаминотрансферазой активное переаминирование с фенилпировиноградной кислотой различных аминокислот, включая треонин, подтверждает мысль Т.Т.Березова, о том, что в тканях млекопитающих оно является главным путем дезаминирования L-аминокислот [16]. Barret [19] отмечает, что млекопитающим, больным уремией и находящимся на низкобелковой диете, давали α-кетокислоты — производные незаменимых аминокислот. Последние синтезировались в организме путем переаминирования. Оказалось, что в виде аминокислот необходимо давать только лизин, для которого отсутствует трансаминаза. В то же время степень использования α-кетокислот для синтеза соответствующих аминокислот различается. Валин, лейцин, изолейцин, метионин и фенилаланин быстро синтезировались путем переаминирования, в то время как гистидин, треонин и триптофан синтезировались в меньшей степени, а синтеза лизина вообще не наблюдали. В свете этого легко объясняется указанный выше следующий факт. Когда кроликов и крыс снабжали рационом с ^{15}N меченным глицином, в треонин эта метка не включалась: в организме млекопитающих треонин из глицина образовываться не может, а переаминированию подвергается лишь очень незначительная часть глицина. В то же время, обнаружение в треонине ^{15}N, введенного в организм крысы с лейцином, есть результат переаминирования, которому активно подвергается лейцин и слабо подвергается треонин [19]. Следовательно, обнаружение в треонине (но не в лизине!) ^{15}N, введенного в организм крысы с лейцином, могло быть только результатом переаминирования.

Стоит также обратить внимание, что Т.Т.Березов в [16] считает, что дезаминирование триптофана осуществляется через разрыв индольного ядра с образованием кинуренина и далее 3-оксикинуренина, которые или подвергаются переаминированию с α-кетоглутаровой кислотой , или распадаются под действием специфической кинурениназы с образованием аланина. Дезаминирование аланина так же происходит путем переаминирования. Но в [19] зафиксировано переаминирование триптофана в количестве, соизмеримом с треонином (по последним данным из всех природных аминокислот только лизин не способен подвергаться переаминированию [19,20]). Из этого можно сделать заключение, что имеется в переаминировании аминокислот видовая специфика :так, у крыс треонин подвергается переаминированию, а у свиней не подвергается(в литературе сведения о переаминировании треонина у свиней полностью отсутствуют), что обусловливает у последних его лимитирующий характер наряду с лизином и триптофаном при характерном для свиней зерновом питании. Исследования, проведенные Mastellar et al. [21], отрицают лимитирующий характер треонина для лошадей, что наводит на мысль о вероятном переаминировании треонина у лошадей.

II. Особенности Превращения Треонина у Человека

Особенности катаболизма треонина у человека. Zhao et al. [22] не удалось обнаружить у человека сколько-нибудь заметного превращения треонина плазмы крови в глицин. Edgar в своей работе [23] проводит сравнение генов треониндегидрогеназы человека и ряда животных и делает вывод, что человек в процессе эволюции утратил способность к синтезу треониндегидрогеназы. Следовательно, необратимый распад треонина под действием треониндегидратазы – единственный путь катаболизма треонина у человека, что согласуется с известным фактом, что треонин для человека – незаменимая глюкогенная аминокислота и что α-кетомасляная кислота – предшественник глюкозы.

Вышеизложенное легко объясняет вред избытка треонина в питании для растущих крысят при его отсутствии для младенцев. Имеющаяся у животных треониндегидрогеназа окисляет (у птиц в большей мере, у млекопитающих-в меньшей) треонин в α-аминоацетоуксусную кислоту, которая самопроизвольно декарбоксилируется в амноацетон, окисляющийся до конечных продуктов в аминоацетоновом цикле. Но в α-аминоацетоуксусную кислоту под действием фермента аминоацетонсинтетазы также превращается глицин путем конденсации с ацетил КоА. Таким образом, у млкопитающих, даже если не происходит превращение треонина в глицин [24,25], эти две аминокислоты конкурируют за окисление в аминоацетоновом цикле. Избыток треонина приводит к гиперглицинемии со всеми вытекающими последствиями. Наиболее грозное осложнение гиперглицинемии-почечнокаменная

болезнь, вызванная окислением глицина в щавелеаую кислоту и отложение ее солей в почках. У людей тоже есть аминоацетоновый цикл и аминоацетонсинтетаза, предназначенные для окисления глицина, но отсутствует треониндегидрогеназа. Следовательно, избыток треонина не может приводить к гиперглицинемии.

Возможность переаминирования треонина у человека. В пользу последнего говорит тот факт, что более полувека назад установлено Институтом питания АМН СССР под руководством академика А.А. Покровского, что для человека из 8 незаменимых аминокислот лимитирующими являются лизин, метионин и триптофан. Стоит кратко рассмотреть катаболизм этих аминокислот у человека, чтобы сравнить с катаболизмом треонина.

Лизин – единственная из природных аминокислот, не способная подвергаться переаминированию, а потому у позвоночных необратим не только распад углеродного скелета лизина (как у всех других незаменимых аминокислот), но и дезаминирование этой аминокислоты. Этим объясняется лимитирующий характер лизина для человека.

В настоящее время установлено, что из существующих в организме позвоночных четырех путей распада триптофана в норме 95% этой аминокилоты необратимо распадается по кинурениновому пути [26, 27]. Поскольку у человека в силу его психической деятельности значительно большая часть, чем у кого-либо из животных, оставшихся 5% триптофана так же необратимо расходуется на образование серотонина и уж совсем незначительная часть декарбоксилируется в физиологически активный триптамин, вряд ли стоит говорить о количестве триптофана, подвергающегося обратимому переаминированию. Следовательно, как и у лизина, налицо лимитирующий характер триптофана.

Метионин же легко подвергается переаминированию, но в отличие от других незаменимых аминокислот для него, кроме переаминирования, характерна и другая реакция обратимого распада- переметилирование. Причем все соединения, получающие у метионина метильную группу, не способны ее отдавать (ремителирование образовавшегося гомоцистеина в метионин осуществляется двумя путями, из которых один требует витамина B_{12} и объясняет кроветворное действие последнего, но рассмотрение этих процессов выходит за рамки статьи; в то же время следует отметить, что витамин B_{12} в животном организме требуется в качестве кофермента только для 2 реакций, которые обе имеют отношение к превращению аминокислот, но к кроветворени**ю**-одна). К таким соединениям относится креатин. Если учесть, что за сутки человек выделяет с мочой в среднем 1,5 г креатинина- продукта необратимого распада креатина, то становится понятным лимитирующий характер метионина.

В то же время никаких свидетельств о лимитирующем характере треонина для человека нет. Это может говорить только об обращении распада треонина, то есть о его переаминировании.

В работе [28] сообщается о кинуренинаминотрансферазе, полученной из печени человека. Этот фермент оказался идентичным серин-пируватаминотрансферазе и аланин-глиоксилатаминотрансферазе. В отличие от серин-пируватаминотрансферазы крыс он катализирует переаминирование ряда аминокислот с пировиноградной, но не с фенилпировиноградной кислотой, причем треонин с ней переаминируется в небольшой степени (схема 2):

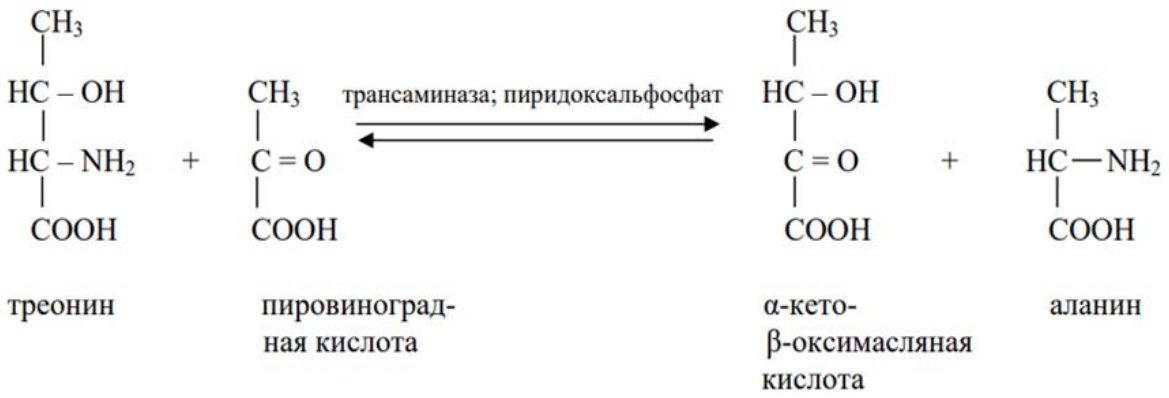

Схема 2: Переаминирование треонина с пировиноградной кислотой в печени человека.

В работе [29] рассматриваются амино адипат аминотрансферазы I и II печени человека. Оба фермента способны катализировать обратимое переаминирование с α-кетоглутаровой кислотой не только α-аминоадипиновой кислоты, но и природных аминокислот, а также кинуренина и орнитина (продукта распада аргинина). Если в отличие от амино адипат аминотрансфераза II амино адипат аминотрансфераза I

не действовала на триптофан и кинуренин, то на остальные аминокислоты, в том числе треонин, действовали оба фермента в одинаково небольшой степени (схема 3):

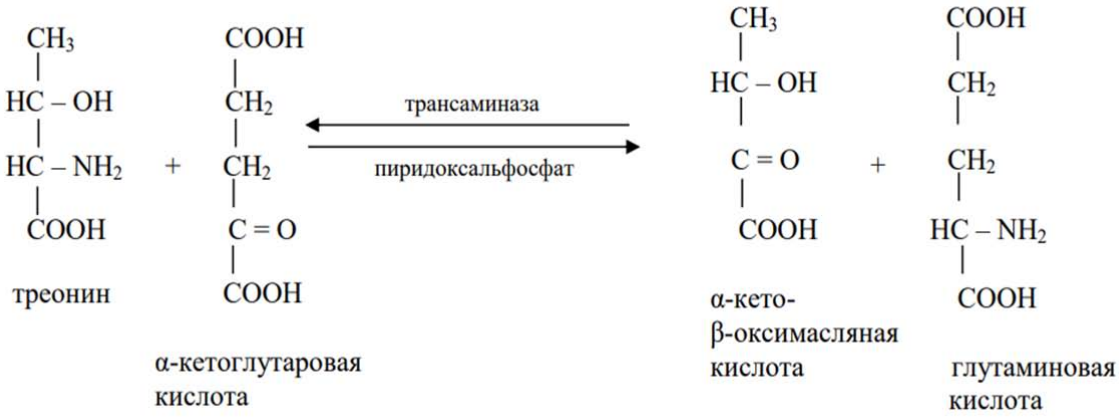

Схема 3: Переаминирование треонина с α-кетоглутаровой кислотой в печени человека.

Работа [30] специально посвящена субстратной специфичности кинуренинаминотрансферазе II, выделенной из мозга человека. Кинуренинаминотрансфераза оказалась идентична аминоадипатаминотрансферазе. Хотя этот фермент активнее всего катализирует переаминирование кинуренина и α-аминоадипиновой кислоты с α-кетоглутаровой кислотой, он обладает широкой субстратной специфичностью и способен переаминировать 16 аминокислот с 16 α-кетокислотами. Среди первых называется и треонин, но среди вторых его кетоаналог не называется. Из этого можно сделать вывод, что α-кето-β-оксимасляная кислота, будучи химически неустойчивым соединением, быстро (но обратимо) восстанавливается в α, β-дигидроксимасляную кислоту с помощью НАДН или НАДФН и соответствующей дегидрогеназы и потому в организме не обнаруживается.

Итак, отсутствие лимитирующего характера треонина для человека при преобладании зерновых в рационе может объясняться только обратимостью начального этапа его катаболизма-переаминирования, которое у человека имеет место. Стоит обратить внимание, что такие незаменимые аминокислоты, как лейцин, изолейцин, валин и фенилаланин не являются для человека лимитирующими, а все они легко подвергаются переаминированию. О высокой способности фенилаланина к переаминированию наряду с лейцином, изолейцином и валином говорит тот факт, что фенилаланин является единственной циклической аминокислотой, которую при уремии заменяют ее α-кетоаналогом в диете [31]. Необходимо отметить, что если для лейцина, изолейцина и валина переаминирование является единственным путем их катаболизма, то катаболизм фенилаланина в здоровом организме в основном заключается в необратимом окислении в тирозин, что никак не противоречит высокой способности фенилаланина к переаминированию. Даже лимитирующая для человека аминокислота метионин, катаболизм которой выражается в необратимой потере серы для превращения серина в цистеин, активно подвергается переаминированию, о чем говорилось выше. что так же позволяет при уремии заменять метионин безазотистым аналогом в диете [29]. Аналогично катаболизм треонина в организме человека заключается в необратимом распаде треонина под действием треониндегидратазы, что никак не противоречит переаминированию некоторого количества треонина.

Но какова же суточная потребность человека в треонине? Давно установлено, что 0,5 г треонина в сутки является минимальной дозой, обеспечивающей азотистое равновесие. Однако количество, гарантирующее азотистое равновесие, у каждой незаменимой аминокислоты должно быть выше в 2 раза этого минимума. Следовательно, для треонина оно будет 1 г в сутки. В то же время академик А.А. Покровский считал , что оптимальное количество белка и незаменимых аминокислот в питании должны не только поддерживать азотистое равновесие, но и обеспечивать сопротивляемость организма к инфекциям и другим вредным агентам внешней среды, способствовать улучшению здоровья и повышению работоспособности. Он предлагал 2-2.7 г треонина в сутки. Примечательно, что ФАО предложено весьма близкое количество треонина в сутки-2,8 г. При этом содержание треонина в большинстве рационов доходит до 3 г в сутки, поэтому говорить о его лимитирующем характере для человека не приходится.

III. Заключение

Если для всех исследованных видов позвоночных, включая человека, незаменимыми являются 8 аминокислот, то лимитирующий характер той или иной незаменимой аминокислоты может отличаться у различных видов. На него могут влиять

различные факторы: содержание данной аминокислоты в рационе, способность к обращению начальных реакций катаболизма, расход той или иной аминокислоты на образование физиологически активных соединений и др. В данной работе на биохимическом уровне показаны причины лимитирующего характера треонина для свиней и отсутствия этого характера для человека, а также проанализированы различные причины лимитирующего характера той или иной незаменимой аминокислоты, что представляет собой большую важность при составлении рационов.

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The Effect of the Medical University Studying on the Eating Habits and the Health of Slovak Students

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Abstract- *Background:* University studying is demanding on the mental as well as the physical side of the organism. However, studying medicine is even more challenging– the study is six years long, and it is tough to remember more information. The study changed the social life of students, and the students didn't have enough time to relax. The examinations of students are full of stress. Stress harms health, especially in the gastrointestinal tract (autonomic nerve system).

Aims: The first study evaluated the relationship between medical university studying (university stress-academic stress) and eating habits and their effect on student's health. We analysed the eating changing during the academic year.

Methods: We made the questionnaire and distributed it to medical students at the Faculty of Medicine in Bratislava, Slovakia. A total of 587 students from the 1st to 6th year completed self-report measures of BMI, academic stress, eating habits and the occurrence or development of digestive problems during the study.

Keywords: *academic stress; eating habit; gastrointestinal disorders; students.*

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Strictly as per the compliance and regulations of:

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Abstract- Background: University studying is demanding on the mental as well as the physical side of the organism. However, studying medicine is even more challenging– the study is six years long, and it is tough to remember more information. The study changed the social life of students, and the students didn't have enough time to relax. The examinations of students are full of stress. Stress harms health, especially in the gastrointestinal tract (autonomic nerve system).

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Results: Our results showed that most respondents were of normal range weight and their eating habits were omnivorous (90%); more than half of the participants had breakfast regularly, and almost half of the students ate junk food a few times per week. Our participants consumed less food but more junk food and energy drinks during the exam period, which can cause obesity and digestive problems.

Conclusion: Our findings confirm that subjective academic feelings of stress play an important role in eating habit changes and in the origin of digestive disorders in our medical students.

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Keywords: academic stress; eating habit; gastrointestinal disorders; students.

I. Introduction

Healthy eating habits are essential in preventing the occurrence of non-communicable diseases (NCDs), including diabetes mellitus, cardiovascular diseases, stroke and cancer. An unhealthy diet and lack of physical activity lead to global health risks. The World Health Organization (WHO) suggests an optimally balanced diet to prevent unhealthy weight gain. Caloric intake should be balanced with its expenditure; total intake of fats should not exceed 30%, and total intake of sugar should not exceed 10% of daily energetic intake, and salts should be kept to a maximum of 5 g per day in order to prevent arterial hypertension, cardiovascular diseases and stroke in adults [1]. WHO's member states signed off on reducing salt intake in the global population by30% by 2025 and agreed to stop growth in the incidence of diabetes mellitus in adults and adolescents and obesity in children by 2025 [2].

Diet is not only affected by social and economic aspects (e.g. income, food prices, individual preferences, cultural traditions and geographical) and environmental factors (including climatic changes) but also by a person's psychological state [3]-[4].

The interaction between stress and eating habits is known. Authors Adam and Epel, 2007) have written about situations when individuals choose unhealthy food under stressful conditions. Stress is also linked with higher consumption of sweets and fats [5], salts[6], but also with a reduction in a well-balanced diet (e.g., meat, fruits, vegetables) [7]. Students are often exposed to stressful, competitive academic conditions, and exam periods have been shown to be extremely demanding psychologically [8]. Negative stress effects could lead to the disruption of a healthy lifestyle and to the occurrence of non-communicable diseases [9].

II. Objectives

It is well known that stress is an essential factor affecting food selection and intake and impacts the origin and development of digestive problems. Stress is

a subjective emotion, and every student can feel the stress of another possibility.

The objectives of this study were to compare the eating habits and their changing of the Medical students studied at the Faculty of Medicine in Bratislava. We analysed the students of all six years of the Faculty studying. We had two hypotheses. The first one was that the eating habits changed during the exam period compared to other parts of the academic year (the exam period vs the holiday or teaching period). The second hypothesis was that students in the first and two last academic years feel more stress than in the other academic years.

III. Materials and Methods

a) Participants

A cross-sectional questionnaire study was conducted during the academic year 2019/2020 before the COVID pandemic began. Five hundred eighty-seven students from all 1948 medical students (137 men (23.3%) and 450 women (76.7%)) from the 1st to the 6th year took part in the study. The Faculty of Medicine in Bratislava has approximately 350 students (1 male to 3 female) every year. We analysed participants' numbers and gender using the R statistics software.

We were asked to complete a health survey questionnaire on their eating habits through social media. We were inspired by the Eating habits Questionnaire [10].

This study was carried out in accordance with the Code of Ethics of the World Medical Association, Declaration of Helsinki (WMA Declaration of Helsinki, 2013), and The University Board approved the project in terms of its ethical aspects.

The inclusion criteria were – actual study at the Faculty of Medicine in Bratislava, Slovak students (not the international students – their eating habits are different from the Slovak eating habits) and the willingness to voluntarily answer the questions. The exclusion criteria were the blank answers.

All questionnaires were completed, and no participants were excluded from the study. The questionnaire was completed anonymously, and the participants were assured of data confidentiality. All participants provided consent to participate after being debriefed about the true nature of the study.

Eating habits during the examination period of the academic year (according to the study plan schedule) and the rest of the academic year (teaching and holiday) were compared.

b) Questionnaire

The questionnaire included four parts. The first contained personal data: gender, age and anthropometric parameters (height and body weight).

The second part of the questionnaire included questions focused on the type of diet; evaluation of eating habits, how often they eat breakfast, where they are used to having lunch, how often they eat snacks (sweet, salty), and how much water they drink daily, how often they drink sweetened drinks and beverages for increasing energy/attention and what nutritional supplements they take.

The next part of the questionnaire focused on changes in body weight, food intake, snacks and energy drinks during the exam period. The final questions concerned the incidence of chronic gastrointestinal tract (GIT) disorders during university studies, changes in the frequency of their occurrence during the examination period, and ways of dealing with GIT disorders.

c) Body Mass Index (BMI)

Body Mass Index (BMI – weight/height^2) was calculated by measuring the weight in kilograms and dividing it by height in squared metres[11].

d) Statistical analysis

For statistical analysis, we used Microsoft Excel and GraphPad Prism 9.4.0 (descriptive statistics, absolute values, means, standard deviation, percentages) and the R Statistics Software for the sample size.

IV. Results

Five hundred eighty-seven of all 1948 students completed the questionnaire –137 men (23.3%) and 450 women (76.7%) – between the ages of 18 and 32 years old (mean ages= 22.16±2.58 years). In accepting or rejecting our hypotheses, we received when more than 50% answers were to one question.

Body Mass Index (BMI) is a statistical index using a person's weight and height to estimate body fat in males and females of any age. It defines a person as underweight, normal weight, overweight and obese. In our study, 60.6 % of men (N=83) had normal BMI values, but more than 31.4% (N=43) were overweight. Among women, 77.2% (N=347) of those monitored had normal BMI; however, 15.1% (N=68) were underweight, and 7.3% (N=33) were overweight. The mean value of men's BMI was 34.3±3.32 kg/m^2, and women's mean BMI was 20.99±2.6 kg/m^2. Most participants (89.6%) ate a well-balanced diet, including meat products, and only 1.4% of the students were vegans. The option pescatarian was selected by 3.4% of students, Lacto-vegetarian by 2.7% and a special diet for health reasons (gluten-free, low histamine, lactose-free, dairy-free) by 3.6% of students.

a) Eating habits outside the examination period

During the semester, more than half of students (56.9%) self-evaluated their healthy eating habits as positive. The other answers are in Table 1.

Table 1: Self-evaluation of having healthy eating habits

Rate	N (%)
Yes	70 (11.9)
Rather yes	334 (56.9)
Rather not	150 (25.5)
Not	33 (5.7)

More than half of the participants - 66.6%- ate breakfast daily, and more than half of the students (56.5%) ate lunch in the canteen (Table 2).

Table 2: Frequency of participants having breakfast and their place of eating lunch

Frequency of having breakfast N (%)	
Every day	391 (66.6)
Irregularly	91 (15.5)
Seldom	55 (9.4)
Never	50 (8.5)
Place of lunch	
Place	N (%)
Canteen	332 (56.5)
Cooking by myself	53 (9)
Restaurant	130 (22.1)
Fast-food restaurant	60 (10.2)
Nocooked meal	12 (2.2)

Almost half of participants ate junk food a few times per week, drank 1 – 2 litres of water per day (48.4%) and rarely drank sweetened drinks (49.5%); the majority of students (75.3%) drank coffee to increase their attention (Table 3).

Table 3: Frequency of eating fast/fried food/or daily drinking habits and kind of drinks

Frequency of eating fast/fried food N (%)	
Never	7 (1. 3)
Seldom	126 (21.5)
2 – 3 times a week	287 (48.8)
Every day	167 (28.4)
Daily water intake (litres/day)N (%)	
Less than 0.5 L	16 (2.7)
0.5 – 1.0 L	138 (23.5)
1.0 – 2.0 L	284 (48.4)
More than 2 L	149 (25.4)
Frequency of drinking sugar-sweetened beverages N (%)	
Never	156 (26.5)
Seldom	291 (49.5)
2 – 3 times a week	134 (22.8)
Every day	6 (1.2)
Drinks to increase energy/an attention N (%)	
Coffee	442 (75.29)
Tea	295 (50.25)
Cola-cola and others	73 (12.43)
Energy drinks	99 (16.86)
None	78 (13.28)

Women took vitamins more often (69.5%) in comparison to men (60%) (Table 4).

Table 4: Dietary supplements

Dietary supplement	Men N (%)	Women N (%)
Vitamins	82 (59.85)	313 (69.55)
Magnesium	55 (40.14)	231 (51.3)
Probiotics	14 (10.21)	72 (16)
Fibre	10 (7.29)	35 (7.77)
Zinc	3 (2.18)	18 (4.0)
Iron	3 (2.18)	7 (1.55)
None	42 (30.65) 85	(18.8)

b) Eating habits during the exam period

Another part of the questions focused on changes in eating habits during the exam period, which is characterised by higher exposure to stress. Half of the women (53.3%) monitored a reduced food consumption under stress exposure (Table 5). Almost half of respondents (47.8%) ate less compared to 25.2% of students who ate more during the exam.

Table 5: Eating habit changes depending on academic year period.

	N (%)
Without changes	158 (27)
During the exam period eating less	281 (47.8)
During the exam period eating more	148 (25.2)
During the exam period eating less – women	240 (53.3)

Almost half of the students (48.8%) eat more junk food during the exam period, and more than half (55.2%) of participants drink more energy drinks. Approximately the same number of students maintained their weight (49.4%) as the lost weight from the study at the medical faculty (50.6%) (Table 6).

Table 6: Changes in eating junk food, drinking energy drinks and changes in body weight depending on the academic year period

Changes in eating junk food depending on academic year period N (%)	
Without changes	280 (47.7)
Increased during exam period	287 (48.8)
Decreased during exam period	20 (3.5)
Changes in appetite depending on academic year period N (%)	
Without changes	250 (42.5)
Increased during exam period	324 (55.2)
Decreased during exam period	13 (2.3)
Changes in body weight from the study at the medical faculty N (%)	
No changes in body weight	290 (49.4)
Weight loss always during exam periods	297 (50.6)

i. *Gastrointestinal problems during exam periods*

The final questions on the questionnaire focused on the incidence of gastrointestinal tract (GIT) disorders and on ways of solving these health issues. Less than half of men (42.3%) and less than one-third of women (26.9%) had no digestive problems. Almost half of the women had diarrhoea (45.55%), abdominal cramps (42.22%) and nausea (37.11%), vomiting (15.33%), gastroesophageal reflux (GER) (7.74%) and constipation (14.8%). Among male students digestive problems occur less often: diarrhoea (26.2%), abdominal cramps (16.7%), nausea (19.7%), vomiting (9.48%), GER (16.05%) and constipation (6.56%) (Table 7).

Table 7: Occurrence of gastrointestinal tract disorders in participants during their study at university

Gastrointestinal disorders	Men (N/%)	Women (N/%)
None	58 (42.3)	121 (26.9)
Diarrhoea	36 (26.2)	205 (45.55)
Abdominal cramps	23 (16. 7)	190 (42.22)
Nausea	27 (19.7)	167 (37.11)
Vomiting	13 (9.48)	69 (15.33)
Gastroesophageal reflux (GER)	22 (16.05)	35 (7.74)
Constipation	9 (6.56)	67 (14.8)

Two-thirds of female students (66.6%) and almost half of male students (46.72%) noted an increased frequency of digestive problems during exam periods(Table 8). Among the students with lower appetite during exam periods, 75.08% had digestive problems more often precisely in this period. Among the students with the same appetite during the academic year, 60.1% noted some form of digestive problems

during the exam period. Half of the respondents (50.6%) with a greater appetite during the exam period also had a higher frequency of digestive problems (Table 6). Among students with a higher energy drink consumption frequency, two-thirds (67.6%) had digestive problems more often (Table 6).

Table 8: The frequency of gastrointestinal tract disorders depends on the academic year period.

	Men (N/%)	Women (N/%)
Without changes	73 (53.28)	168 (37.34)
Increased during exam period	64 (46.72)	282 (66.66)
Decreased during exam period	-	-

Among students with digestive problems, 63.2% did not solve their problems, 13.28% decided on a change of diet, 11.75% took over-the-counter drugs, 10.2% visited a doctor, and 9.2% drank digestive tea (Table 9).

Table 9: Solving gastrointestinal tract disorders by the participants

	N (%)
Did not solve the GIT disorder	371 (63.2)
Diet	78 (13.28)
Taking an over-the-counter drug	69 (11.75)
Drinking digestive tea	54 (9.2)
Visiting a doctor	60 (10.2)

V. Discussion

This study monitors the changes in diet in Slovak medical students under stress (academic stress) exposure during the exam period of the academic year before the Covid pandemic. Most respondents had normal weight, but one-third of the men were overweight. In Polish, Hungarian and American students, a higher incidence of overweight in men was also noted in comparison to women [12]–[14]. On the other hand, the obesity of Iranian students has proportionally reversed: 22.5% of Iranian women were overweight in comparison to 7.3% of Slovak women, and 7.9% of Iranian men were overweight in contrast to 31.4% of Slovak men [15]. The prevalence of obesity among university students was caused by their socio-economical situation, as well as ethnicity, education, income, culture, eating and exercise habits. The higher incidence of overweight in male students in comparison to women may also be caused by different amounts of muscle mass; thus, a better indicator of obesity is measuring the amount of fat and its distribution in the body [16].

The majority of Slovak medical students reported eating a varied diet that included animal products (an omnivorous type of diet), and only 1.4% were vegans. Studies show that vegans have a low body mass index (BMI) and low cholesterol levels in blood plasma [17]. The Mediterranean diet appears to be ideal for preventing cardiovascular diseases and obesity [18], [19]. We can speak about the Mediterranean diet as it is composed of extra virgin olive oil, fresh fruit, vegetables, cereals, nuts, legumes, fish, meat, dairy products, red wine and low amounts of eggs and sweets [20].

In our study, more than half of the participants had breakfast regularly, the same as Chilean students, who ate breakfast 5 – 7 times per week [21] The percentage distribution of the diet during the day affects body composition. Chilean students with a low-fat body percentage consumed almost 19% of their daily intake at breakfast, whereas students with a high body fat percentage consumed only 8.9% of their daily intake for breakfast [22].

Eating junk food can increase body weight. In our study, 48.8% of students consume junk food a few times per week. Caso et al., [23] and [24] confirmed that overweight students eat junk food more often and mainly during times of negative emotions (sadness, anger, fear, academic stress) in comparison to students with normal weight [23].

Slovak and American students drink coffee to improve concentration. In addition, American students drink coffee to increase attention (79%), because of its taste (68%), for socialising (39%), to improve physical energy (27%) and mood (18%), and to relieve stress (9%) [25].

Stress is defined as the state of affairs arising when a person relates to situations in certain ways. People are not disturbed by conditions but by how they appraise and react to situations. In general, a person experiences stress when demands exceed a person's coping abilities, resulting in reactions, such as disturbances of cognition, emotion and behaviour, which can adversely affect well-being. The majority of students experience academic stress [26], and the exam period appears to be the main source of stress [27]. Academic stress refers to the stress associated with the academic environment, writing tests, performing difficult cognitive tasks or being evaluated [28] The current literature offers inconsistent findings regarding gender relative to perceived levels of stress [29]. Ng et al., [30] found that females were more likely to feel as though they experienced higher levels of stress, which was in agreement with Thawabien [31]. In addition,

female students reported more stress-related issues, such as low self-esteem, pressure from exams and depression [31]. However, no gender differences in coping with stress were found by Donaldson [32].

Exposure to stress factors can lead to gastrointestinal problems [33]. Knowles et al.,[34]monitored significantly more digestive problems, increased levels of cortisol and decreased amounts of bacterial strains before exam periods. These findings play an important role in the prevention and therapy of digestive problems during stress periods. Increased levels of cortisol are characteristic of chronic stress [35]and there is a relationship between abdominal obesity and higher levels of cortisol [36]. Activation of the sympathetic adrenal medullary system, with the release of catecholamines (epinephrine and norepinephrine) during the acute stress response, leads to a reduction of appetite [35].

Stress can cause an increased or decreased appetite. Chronic stress is characterised by a preference for junk food (with high levels of fat and sugar), and studies show that chronic stress can lead to overweight, mainly in the male population [35]. In our study, half of the respondents consumed less; however, during the examination period, they consumed more junk food. Caso [23] confirmed that academic stress is connected with higher consumption of junk food. Oliver [7] found that approximately the same number of students ate less (38%) or more (42%) food during examination periods. In contrast, the appetite of English students did not change; there were changes in the macronutrient intake ratio, but total calorie intake did not change during exam periods [37]. In our study, half the participants (55.1%) drank energy drinks to improve their concentration, and approximately the same number of students (50%) lost weight or did not change in weight during academic stress.

In general, stress can increase or decrease appetite, and a well-balanced diet and BMI can also be affected by gender [6]. Stress-eaters (people who eat more under negative emotions, such as fear and sadness) more often choose a high fat and/or high carbohydrate diet under stress conditions [38].

Brain signals can affect the motor, sensory and secretory modalities of the gastrointestinal tract; however, gut signals are affected by emotional behaviour. Stress and pain modulate this system using the nervous, endocrine and immune systems. As a result, the gut-brain axis has changed to the microbiota-gut-brain axis. Therapy with probiotics appears to be suitable for abdominal dysfunction caused by stress. Kato-Kataoka et al. [39] monitored the effect of the probiotic bacteria *Lactobacillus case i*s train Shirota on abdominal dysfunction in a double-blind study composed of students before an exam period. They monitored the positive effect of daily intake of probiotics. They found that whereas cortisol levels were significantly increased in the placebo group, in the group with probiotic intake the amount of bacterial strains in the gut microbiota was significantly increased. The percentage of members of the family *Bacteroidaceae* was significantly reduced.

Exposure to stress factors plays a key role in the development of visceral pains (e.g. functional gastrointestinal diseases; irritable bowel syndrome [40]. In our study, almost 60% of students had digestive problems during the examination period, and women, in particular, suffered from diarrhoea, abdominal cramps, nausea and constipation. Yildirim et al. [41] confirmed the interaction between stress and constipation in Turkish medical students. They also monitored the percentage increase in the number of students with constipation depending on the year of study. Abdominal aroma and meridian massage positively affect constipation and frequency of defecation in female students. Application of abdominal massages leads to a reduction of drug intake and can help to release stress during an exam period [42]. Stress appears to be one of the factors affecting the origin of irritable bowel syndrome. The prevalence of irritable bowel syndrome in Chinese students was 15.7%, and the most common symptoms were changes in stool consistency, frequency of defecation and the presence of abdominal pain released with defecation. The symptoms appeared more often in female students. Psychological and psychosomatic symptoms of affairs and depression were more common in patients with irritable bowel syndrome [43]; therefore, increased demands on students' psyche during exam periods could lead to digestion problems. Polish students with a lack of physical activity repeatedly suffered from digestive problems and were frequently more absent from school because of abdominal pains than students with a sufficient amount of physical activity [44].

a) Limitations

The present study has several limitations. Data were collected and evaluated from Slovak medical students, who filled answers themselves; thus, the participants in our study do not necessarily represent the nutrition of Slovak medical students in general.

VI. Conclusions

In conclusion, this is the first study dealing with the nutrition issues of Slovak medical students depending on the period of the academic year. Our results suggest that in higher exposure to stress, mainly during exam periods, the participants consumed less food but more junk food and energy drinks, leading to overweight or obesity. We confirmed the assumption that academic stress plays an essential role in the origin of digestive problems, which could be decreased by nutrition education and effective approaches to cope with stress during university life.

Author Contributions

AG and ZJ conceived and designedthe study. DF and AG were responsible for data collection. ZJ, DF, ZF, JK and AG analysed the datasets. ZJ, DF, ZF, JK and AG wrote the primary draft of the manuscript. All authors critically reviewed the manuscript and have approved the final article.

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Animal Model for Bone Implant in Swine

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Abstract- *Objective:* The main purpose of this study was to develop an Animal Model that could accommodate evaluation experiments for cortico-spongy implants in the areas of orthopedics, dentistry and neurology.

Methodology: On the anteromedial surface of the proximal third of the tibia of pigs, we have a cortico-spongy area that is basically subcutaneous. This region proved to be ideal for this purpose.

Results and discussions: The pig is already a well-known animal for testing biomaterials in bones because its boneregeneration rate (1.2 and 1.5 mm/d) is comparable to that of humans (1.0 and 1.5 mm/d).

Conclusion: The Animal Model was formatted, which proved to be simple and reproducible.

Keywords: *porcine animal model, cortical spongy bone, proximal tibia.*

GJMR-G Classification: *DDC Code: 618.190592 LCC Code: RD539.8*

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Animal Model for Bone Implant in Swine

Marco Antonio Schueda [α], Thiago Fuchs [σ], Welington Martins Belo [ρ], Lara Thaís Marcon [ω], Leide Daiana Bay [¥] & Fernanda Heidemann [§]

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I. Introduction

With the evolution of science and technology, especially in the field of medicine, the use of Animal Models for the evolution of techniques and treatments have become of paramount importance. Galen (129-210 AD), a precursor of experimental medical research with the use of animals inspired supporters of this study plan, but it was Claude Bernard in 1865, who launched the principles of its use in the work "Introduction to the Study of Experimental Medicine" establishing the rules and principles for such. From then on, the significance of using animal models gained more attention and relevance[1].

In orthopedics, an anatomy closer to the human anatomy is required, as well as a similar pathophysiological and histological composition, in order to present relevant results[2].

Several animal test models, such as rats/mice[3,4,5], rabbits[3,6,7], dogs[3,8,9], sheep[3,10,11], goats[3,12,13], and pigs[3,14,15], have been developed to simulate environment and physical conditions testing the biocompatibility of human bone substitute biomaterials "in vivo". In order to simulate various orthopedic situations, many defect sites that have been explored, such as calvaria[3,16,17], femur/tibia[3,18,19] and ulna[3,20,21,22].

Factors should be considered when selecting a specific animal species as a test model. First, the chosen animal model should clearly demonstrate significant physiological and pathophysiological analogies compared to humans. Second, it should assess whether it is possible to operate and observe a multitude of study objects after surgery over a period of time[23]. Other selection criteria include acquisition and care costs, availability of the animal, acceptability by society, tolerance to captivity, and ease of housing24. According to the international standard, we should also consider the size of the implant test specimens, number of implants per animal, intended test duration, and possible differences between species when correlated with biological responses[25].

In the present study, pigs were preferred as anatomical models. This choice was initially due to the greater morphological/anatomical similarity with humans, besides the ease of releasing them for in vivo study. Following the choice of pigs as animal models, we specified the need for anatomical regions whose histology presented cortico-spongy regions. This histology is present in metaphyses of long bones, such as the femur and the tibia. We limited the study to the tibia, since it has a triangular shape and the anteromedial region of its proximal third is basically subcutaneous, thus facilitating its approach.

A new procedure that will facilitate the evaluation of biocompatibility and recovery after cortico-cancellous implants in Medicine and Veterinary Medicine in the specialties of Orthopedics, Traumatology, Dentistry and Neurology will be presented in this work.

II. Material and Methods

Work performed in the Veterinary Surgical Center of the Mafra Campus of UNC

– Fundação Universidade do Contestado (Figures 1 A and 1 B)

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Fig. 1 A: UNC Veterinary Surgical Center (Campus Mafra - SC)

Fig. 1 B: Procedure room with specific lighting, instrumentation and anesthesia equipment.

Preparation of materials and surgical specimen (Figure 2)

Fig. 2: Prepared materials and part

After localization of the knee joint with 2.0 Kurschner wire, the anteromedial surface of the proximal tibia is accessed with a 3.5 cm incision with a number 23 blade up to the periosteum (Figures 3 A and 3 B).

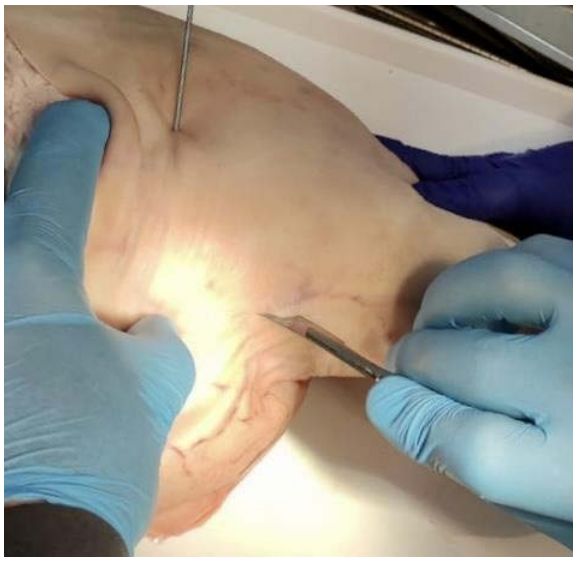


Fig. 3 A: After passing the Kirschner wire in the knee joint, a 3.5 cm incision is made on the anteromedial surface of the proximal third of the tibia.

Fig. 3 B: Incision up to the periosteum with incision and exposure of the bone surface.

As a guide we use 2.0 kurschner wire and then we drill with a 6 mm drill (Figures 4 A and 4 B).

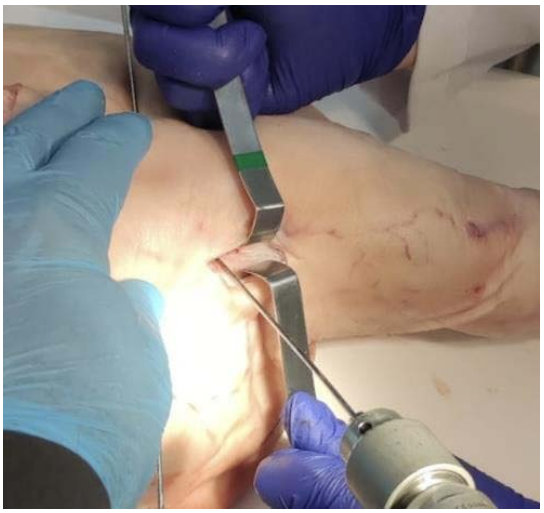


Fig. 4 A: Drilling with kurschner 2.0 guide wire on tibial anteromedial surface

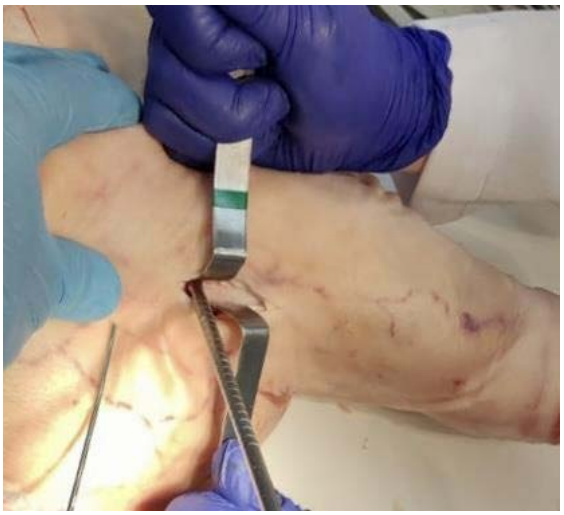


Fig. 4 B: Following the guide wire, it is drilled 20 mm deep with a 6 mm drill.

Following the guide wire, an 8 mm x 20 mm screw is inserted by turning it up to the tibial cortical surface (Figures 5 A and 5 B).

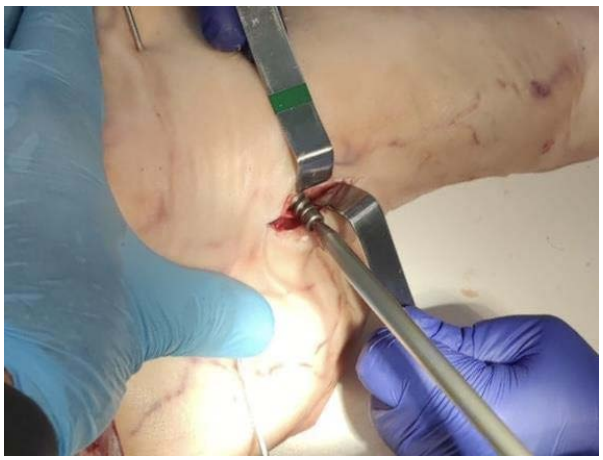


Fig. 5 A: 80X20 mm implant is screwed following the guide wire

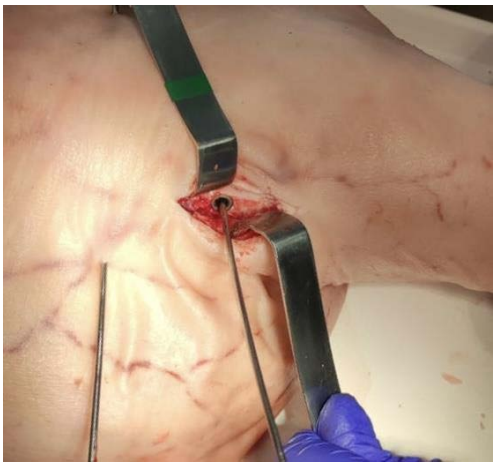


Fig. 5 B: Until aligned with the cortical surface of the Tibia

Repeat the procedure at 25 millimeters from the center of the first wire and at 50 millimeters fixing two more screws (Figures 6)

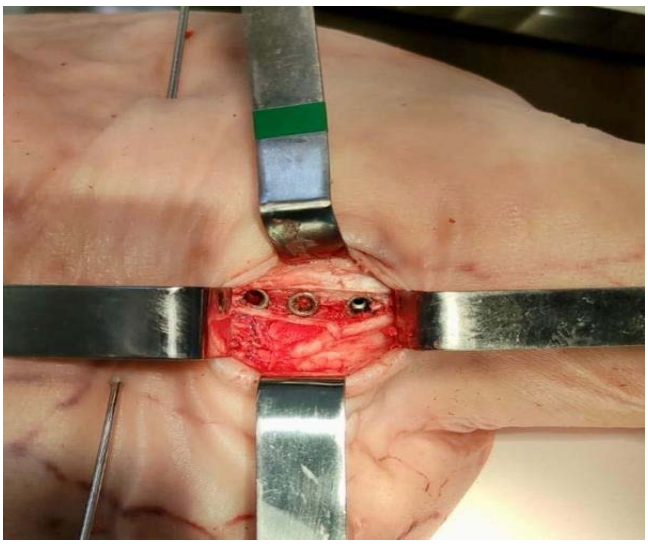
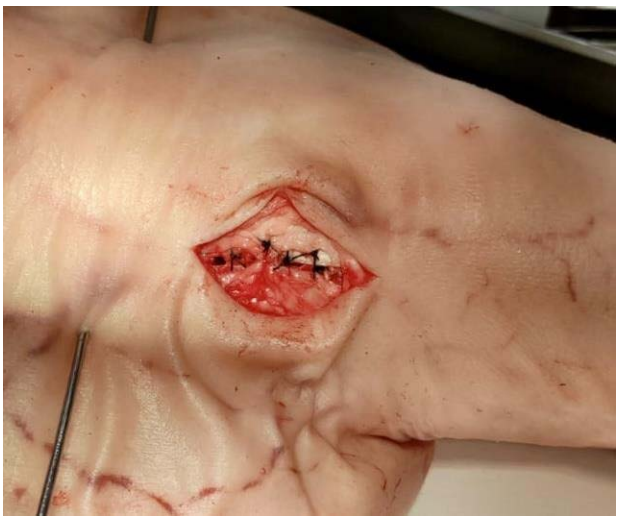


Fig. 6: Fixation of the three interference screws in the medial Antero surface of the proximal third of the tibia

After fixing the screws, the periosteum is sutured, the intra-articular thread is removed, and the skin is sutured. (Figures 7 A and 7 B)



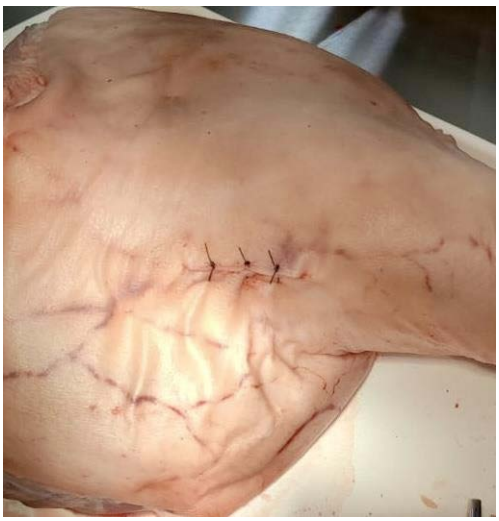


Fig. 7 A: Periosteal suture Fig. 7 B - Skin suture

Radiographic study was done post procedure to visualize the positioning of the implants (Figures 8 A in Posterior Antero and 8 B in Profile)

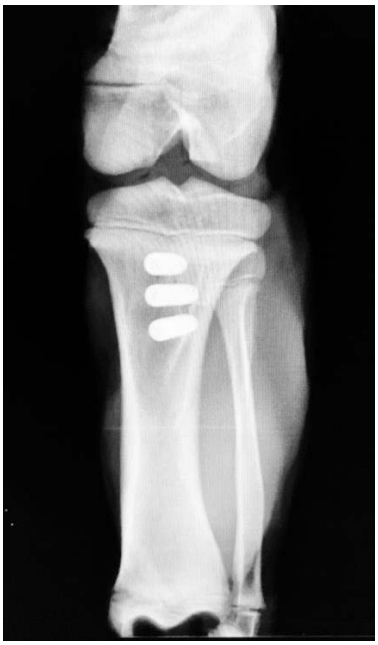


Fig. 8 A: AP X-ray (Posterior Anterolateral) - Porcine tibia

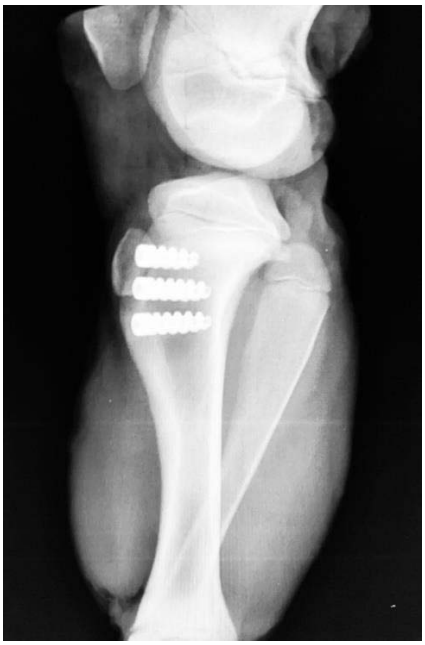


Fig. 8 B: Profile X-ray study - Porcine tíbia

III. Results and Discussions / Theoretical Framework

Pigs are considered close representative models with regard to bone anatomy, morphology, healing capacity, remodeling, mineral density, and concentration[26,27]. Similarities in femur cross-sectional diameter and area have been found between humans and pigs[28]. Pigs have a lamellar bone structure similar to humans[29]. However, pigs have a denser trabecular network that is considered to be intricate. They are difficult to handle, noisy, and aggressive; therefore, pigs are often overlooked in favor of more receptive species such as sheep and goats[30,31]. In addition, the length of tibias and femurs in pigs is relatively short, which cannot meet the special needs of human implants. The pig was the animal chosen for critical size defect models to test bone substitute biomaterials because its bone regeneration rate (1.2 and1.5 mm/d) is comparable to that of humans (1.0 and 1.5 mm/d)[22].

Commercial pigs are generally considered undesirable for orthopedic research because of their high growth rates and very high body weight. The development of minipigs and micropigs has overcome this problem to some extent. However, in our region, these animals are easy to obtain and manage, making the application of this Animal Model quite easy.

IV. Conclusão

It was possible to design the Animal Model in pigs for cortico-spongy implants that is simple and reproducible to execute.

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Malocclusion in a Rabbit – Case Report

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Abstract- Dental malocclusion is a highly prevalent pathology in domestic rabbits, usually resulting from food management errors. The present study aims to report the procedures used in a rabbit treated at the Veterinary Hospital in João Pessoa, Paraíba, affected by this pathology and to highlight the clinical characteristics and the treatment of occlusal adjustment, made through tooth wear, presented by this species in this condition.

Keywords: *rabbit, malocclusion, occlusal adjustment, tooth wear.*

GJMR-G Classification: *NLM: SF997.R3*

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Malocclusion in a Rabbit – Case Report

Má Oclusão Em Um Coelho – Relato De Caso

João Guilherme Coelho de Souza

Resumo- **A má oclusão dentária é uma patologia de alta prevalência em coelhos domésticos, normalmente proveniente de erro de manejo alimentar. O presente estudo tem como objetivo relatar os procedimentos utilizados num coelho atendido no Hospital Veterinário em João Pessoa, Paraíba, acometido desta patologia e destacar as características clínicas e o tratamento de ajuste oclusal, realizado através do desgaste dentário, apresentado por esta espécie nessa condição.**

Palavras-Chave: coelho, má oclusão, ajuste oclusal, desgaste dentário.

Abstract- **Dental malocclusion is a highly prevalent pathology in domestic rabbits, usually resulting from food management errors. The present study aims to report the procedures used in a rabbit treated at the Veterinary Hospital in João Pessoa, Paraíba, affected by this pathology and to highlight the clinical characteristics and the treatment of occlusal adjustment, made through tooth wear, presented by this species in this condition.**

Keywords: rabbit, malocclusion, occlusal adjustment, tooth wear.

I. Introdução

A má oclusão dentária é uma patologia de alta prevalência (aproximadamente 70%) em pets não convencionais como coelhos e demais roedores FECCHIO, Roberto (2022), com manifestações clínicas tardias, sinais inespecíficos, dificil identificação por parte dos proprietários e médicos veterinários, que muitas vezes, encontram-se despreparados para tais acontecimentos. Objetivou-se com este trabalho evidenciar as características clínicas e os procedimentos utilizados no tratamento de um caso de má oclusão dentária em coelho, atendido no Hospital Veterinário em João Pessoa, Paraíba. Foi realizado exame clínico e exames de imagem (estudo radiográfico do crânio) e indicado o procedimento odontológico de ajuste oclusal, que consta no desgaste dentário, imprescindível a pacientes dessa espécie acometidos por esta patologia. Foi também indicada a correção da dieta oferecida regularmente ao animal.

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II. Revisão de Literatura

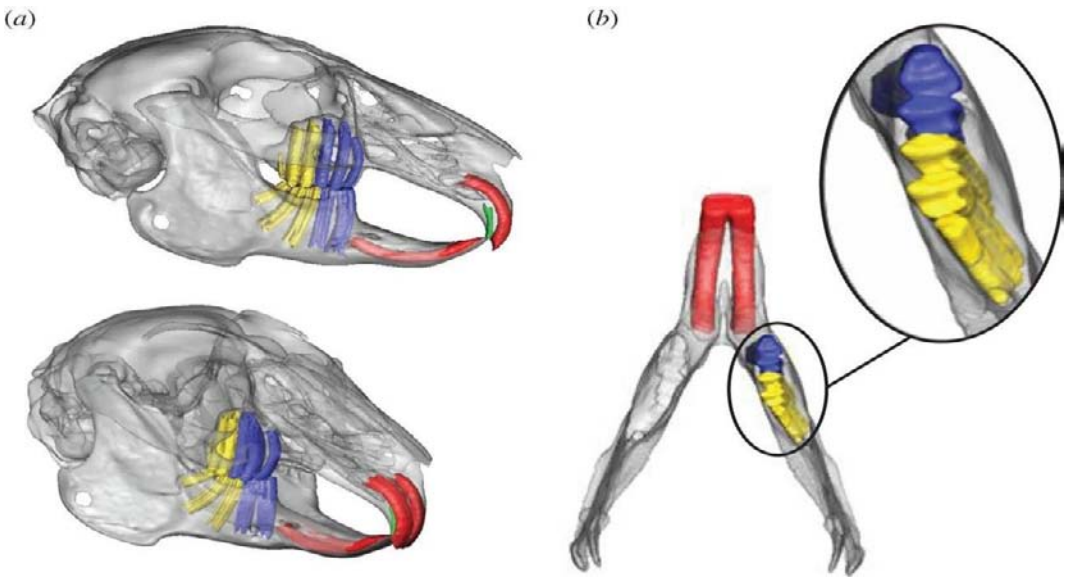
Lagomorfos (coelhos) e os demais roedores são animais de estimação cada vez mais populares. E neles encontramos uma alta incidência de problemas bucais/dentários que o médico veterinário precisa ser capaz de identificar e gerenciar. A maioria dos problemas está relacionada às peculiaridades anatômicas de sua dentição em combinação com a má criação, ou seja,alimentação não abrasiva resultando em desgaste anormal e má oclusão GORREL, Cecilia (2013). A má oclusão dos incisivos, portanto, é comum em coelhos segundo VESTRAETE, Frank (2005). Se esta condição ocorre como uma entidade isolada em idade precoce, provavelmente tem origem genética, mas quando a má oclusão dos incisivos se dá em animais mais velhos geralmente é secundária e/ou ocorre concomitantemente com a má oclusão pré-molar molar. FECCHIO, Roberto (2022) afirma ser uma patologia de alta prevalência (aproximadamente 70%), com manifestações clínicas tardias, sinais inespecíficos, dificil identificação por parte dos proprietários e médicos veterinários, que muitas vezes, encontram-se despreparados para tais acontecimentos.

Os coelhos, lebres e pikas são mamíferos pertencentes à ordem dos lagomorfos, são herbívoros que em seu habitat natural pastejam grande quantidade de vegetação dura, energeticamente pobre, bastante fibrosa (rica em sílica, hemicelulose, celulose e lignina), seus movimentos mastigatórios vigorosos e constantes promovem o desgaste dentário alto. Mas a domesticação afetou sobremaneira a dieta desta espécie pois hoje de acordo com FECCHIO; Roberto (2022) a dieta encontra-se por vezes desbalanceada, havendo uma oferta excessiva de ração peletizada (com alto valor energético, baixo teor de fibras), muito oferecimento de petiscos (em forma de frutas e verduras) e pouca oferta de alimentos fibrosos gerando perda nos movimentos mastigatórios e baixa taxa de desgaste dentário além de disfunções gastrointestinais FERREIRA, Larissa (2020).

A dentição dos coelhos é o tipo heterodonte, todos os dentes são elodontes (crescem por toda a vida) o número de dentes é 28 e tem fórmula dentária: I2/1, C0/0, P3/2, M3/3. O músculomasseter é o principal músculo da mastigação; o músculo temporal é pequeno; portanto, o processo coronoide é pequeno; o crânio é fenestrado; existem grandes processos semelhantes a asas nos ossos frontais; há um grande

forame incisivo; há uma ponte palatina entre os pré-molares formados por porções dos ossos maxilar e palatino; possuem grandes bolhas timpânicas EMILY, Peter (2021). A mandíbula superior do coelho contém três pré-molares e três molares, enquanto a mandíbula inferior possui dois pré-molares e três molares através da visualização das raízes dosdentes a partir dos dados de micro tomografia computadorizada observou-se que os pré-molares da mandíbula têm raízes predominantemente orientadas verticalmente (Fig. 1). Em comparação, enquanto as raízes molares na maxila também são verticais, elas têm uma orientação mais posterolateral na mandíbula. WATSON, Peter J. (2014). A altura média da coroa dos incisivos mandibulares e maxilares em coelhos domésticos adultos é de 6,0 mm. A profundidade de sondagem periodontal saudável normal dos incisivos inferiores em coelhos é de 5 a 6 mm. Os pré-molares e molares são agrupados como uma unidade funcional com uma superfície oclusal relativamente horizontal com dobras transversais de esmalte para triturar e picar alimentos fibrosos difíceis. As dobras de esmalte correspondem à invaginação profunda do esmalte no lado palatino dos dentes da bochecha maxilar e no lado vestibular dos dentes da bochecha mandibular. As dobras do esmalte são preenchidas com material semelhante ao cemento e são visíveis do lado de fora como sulcos de desenvolvimento. O esmalte periférico é mais espesso nas superfícies linguais dos dentes maxilares e nas superfícies vestibulares dos dentes maxilares. O músculo masseter é muito maior que o músculo temporal, e o processo coronóide é pequeno em comparação com o dos carnívoros (como uma adaptação da ingestão de alimentos fibrosos e duros). A oclusão é anisognata – o arco maxilar é mais largo que o arco mandibular. O plano oclusal é inclinado aproximadamente 10° em direção à horizontal. A forma da articulação temporomandibular permite principalmente movimento lateral considerável, mas muito pouco movimento rostrocaudal. Os incisivos inferiores ocluem entre o primeiro e o segundo incisivos superiores VESTRAETE, Frank (2005).

O crescimento excessivo do dente geralmente resulta em má oclusão. As complicações da má oclusão incluem: Traumatização dos tecidos moles orais (bochechas, língua) pelos dentes crescidos; supercrescimento apical com penetração resultante dos dentes superiores nas cavidades oculares e/ou seios nasais; supercrescimento apical dos dentes mandibulares com penetração resultante da borda ventral do osso alveolar na mandíbula; abscesso retrobulbar e/ou facial; incapacidade de fechar a boca; incapacidade de mastigar (movimento de corte lateral em lagomorfos) segundo GORREL, Cecilia (2013); obstrução do canal lacrimal que de acordo com TEIXEIRA, M. (2021) os lagomorfos possuem uma anatomia de crânio que os predispõe justificando a alta prevalência da condição na espécie. Os coelhos possuem apenas um ponto lacrimal, que se situa na margem palpebral inferior. Este ponto leva a um pequeno canalículo que dilata e dá origem ao ducto nasolacrimal que desemboca nas narinas. O ducto nasolacrimal é tortuoso passando através dos ossos lacrimais e maxilares e correndo próximo ao ápice dos dentes molares e incisivos maxilares. O ducto nasolacrimal dos coelhos também apresenta uma mudança abrupta de diâmetro em seu trajeto, especificamente na curvatura medial ao passar através da incisura infratroclear e do forame do osso lacrimal e entrar no canal nasolacrimal ósseo, localizadomedial ao osso maxilar junto com o recesso maxilar. Além disso, a relação íntima do ducto nasolacrimal com as estruturas dentárias dos coelhos deve ser ressaltada, já que seu trajeto é próximo das coroas de reserva dos dentes incisivos e molares maxilares. Em um estudo com 28 coelhos com dacriocistite, 50% dos animais apresentavam doença dentária como causa de base. A dacriocistite secundária a doença dentária se dá pela projeção do ápice dentário em sentido dorsal dos dentes maxilares. Esses dentes são intimamente localizados próximos ao trajeto do ducto nasolacrimal nos coelhos e a projeção dos ápices dentários pode levar a uma irritação mecânica do ducto gerando inflamação, estenose parcial e até obstrução completa FERREIRA, Larissa (2020).

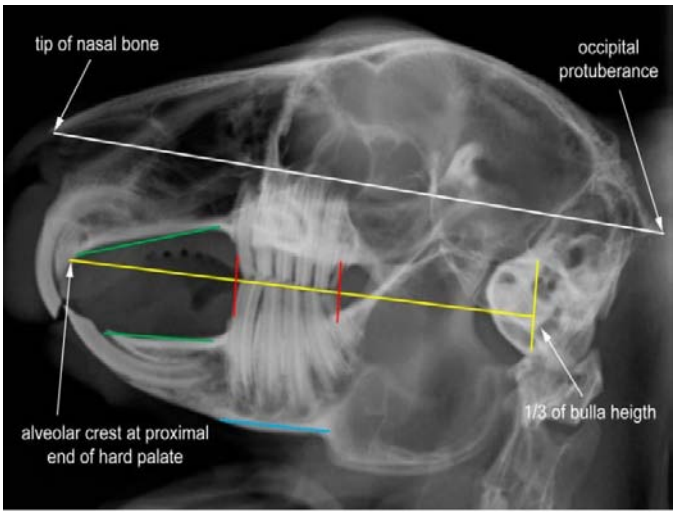


Fonte: rsif.royalsocietypublishing.org J. R. Soc. Interface 11: 20140564

Fig. 1: Visualização da raiz do dente molar e incisivo através dos dados da microtomografia computadorizada: (a) vista lateral e oblíqua; (b) close-up das raízes dos dentes da mandíbula. Os pré- molares (azul) têm raízes predominantemente orientadas verticalmente, enquanto os molares (amarelo) têm uma orientação mais póstero-lateral.

O exame da cavidade oral de roedores e coelhos domésticos faz parte de todo exame físico e é muito importante para o diagnóstico da síndrome do desgaste dentário inadequado, que leva ao hipercrescimento dentário e consequentemente a problemas sistêmicos, no entanto, o exame oral em um animal consciente fornecerá apenas informações limitadas. O exame oral definitivo só pode ser realizado sob anestesia geral SOARES, Helena Baggio (2018). Tem havido inúmeras publicações nas últimas duas décadas descrevendo alterações patológicas específicas observadas nas diferentes espécies. Muitos deles apontam a importância de um exame radiográfico completo do crânio usando múltiplas visualizações e dão alguns conselhos úteis sobre o tratamento, como o uso de linhas de referência anatômicas que simplificam muito a explicação dos problemas bucais aos proprietários do animal (Fig. 2 e 3), pois eles podem ver claramente a extensão das alterações. Isso permite que eles entendam melhor as recomendações de tratamento e quaisquer complicações associadas BOEHMER, E (2009). Ao incluir a radiografia como ferramenta diagnóstica, o número de pacientes relatados que sofrem de má oclusão causada por diferentes alterações periodontais e dentárias patológicas chega a até 88% da população clínica geral BOEHMER, Christine (2020).

Os estudos radiográficos da cabeça incluem projeções radiográficas laterais, dorsoventral, rostro caudal e, quando necessário, incidências oblíquas. Para projeções laterais e oblíquas, o paciente é colocado em decúbito lateral. Para projeções dorsoventral, o paciente é posicionado em decúbito ventral. Pequenas cunhas de espuma radiolúcida podem ser necessárias para um posicionamento preciso antes que a cabeça seja presa ao cassete. As projeções radiográficas oblíquas requerem rotação de 30 graus ou menos da projeção lateral reta. As projeções oblíquas são descritas pelo ponto de entrada do feixe de raios-x até o ponto de saída. Diretrizes de exposição radiográfica para roedores, coelhos usando técnica de mesa e distâncias de filme focal de 40 polegadas (102 cm) para extremidades e 38 polegadas (97 cm) para todos os outros estudos.Aplica-se de 6-7,5 mAs e de 54-58 kV para pequenos a grandes coelhos SILVERMAN, Sam (2005).



Fonte: E. Boehmer; D. Crossley: Objective interpretation of dental disease in small mammals

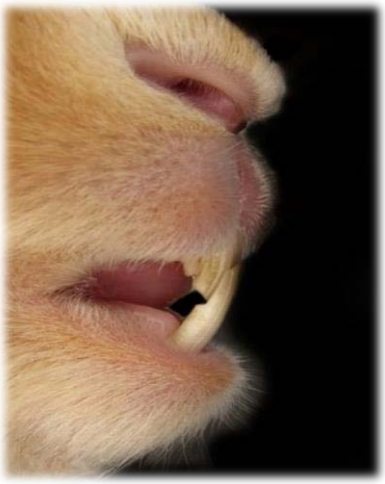
Fig. 2: Linhas de referência anatômicas radiográficas de um coelho clinicamente saudável. Vista Latero Lateral.

Fig. 3: Linhas de referência anatômicas radiográficas de um coelho clinicamente saudável. Vista Dorso Ventral.

Para que haja um equilíbrio da saúde oral desses animais, a taxa de crescimento dentáriodeve ser mais ou menos igual à taxa de desgaste dentário; assim, os dentes se mantêm sempre do mesmo tamanho e o animal preserva a saúde oral. GORREL, Cecilia (2013) citando Wiggs & Lobprise, 1995 diz que os incisivos são usados principalmente em um movimento de fatiamento lateral, de modo que cortam mais ou menos seus alimentos em pedaços menores e apreensíveis. Os incisivos superiores grandes crescem a uma taxa média de 2,0 mm por semana e os incisivos inferiores a uma taxa de 2,4 mm por semana. As técnicas para tratamento e controle consitem nacirurgia de ajuste oclusal, que de acordo com FECCHIO, Roberto (2022), precisa ser feita com equipamentos específicos para a espécie (uma vez que coelhos possuem uma prega de mucosa localizada logo atrás dos incisivos que divide a cavidade oral em duas câmaras: anterior e posterior), proceder o desgaste dentário fazendo odontosecção dos incisivos com brocas específicas (carbide esférica nº 8, carbide cônicas nº 701, 702, 703 e brocas cilindricas diamantadas ou disco de corte), sempre com proteção adjacente aos tecidos moles, irrigação e resfriamento das mesmas, além de luxadores curvos para os casos de exodontia e posterior correção do manejo alimentar.

III. Relato de Caso

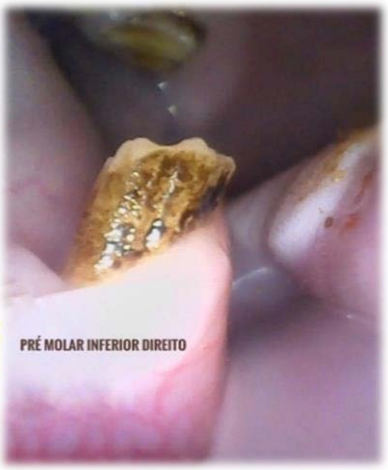
Foi atendido no dia 21 de Agosto de 2021, no Hospital Veterinário Jefferson Ricardo em João Pessoa, Paraiba uma coelha de aproximadamente 01 ano de idade, fêmea, inteira, pesando 1,9 kg, com histórico de ter sido perseguida no dia anterior por um cachorro no condomínio ondemora, e desde então ficou quieta e sem querer comer. O animal apresentava-se tranquilo ao exame físico, permitindo a manipulação, parâmetros fisiológicos sem alterações dignas de nota. Na avaliação clínica específica da cabeça constatou-se epífora no olho esquerdo, fato este que na anamnese a tutora relatou já estar presente antes da queixa principal ter ocorrido, a cavidade oral foi examinada com a visualização rostral do desalinhamento dos incisivos por meio do simples afastamento dos lábios (Fig. 4 e 5) e em seguida o animal foi deitado em decúbito dorsal no colo da tutora e utilizou-se um espéculo vaginal metálico (para a abertura das bochechas) e uma micro câmera (para registrar a imagem intra oral) (Fig. 6 e 7) onde visualizou-se a formação de pontas dentárias no sentido lingual dos pré molares mandibulares direito e esquerdo. Ao ser questionada sobre a dieta, a tutora relatou que era exclusivamente ração peletizada e frutas (banana, pera e eventualmente pepino e couve).



Fonte: Arquivo pessoal do autor

Fig. 4: Desalinhamento oclusal dos incisivos inferiores. Vista Rostral.

Fig. 5: Desalinhamento oclusal dos incisivos inferiores. Vista Lateral.



Fonte: Arquivo pessoal do autor

Fig. 6: Ponta dentária em pré molar inferior esquerdo (seta vermelha). Vista rostral intraoral.

Fig. 7: Pré molar inferior direito. Vista rostral intraoral.

Foi realizado a coleta de sangue para exames laboratoriais e o paciente foi encaminhado para estudo radiográfico do crânio, onde foi requisitado três projeções a saber: rostro caudal, ventro dorsal e latero lateral (Fig. 8, 9 e 10) e analisado de acordo com BOEHMER, E, 2009 as linhas de referência anatômica para a espécie fechando o diagnóstico de má oclusão dentária pordesgaste ineficiente.

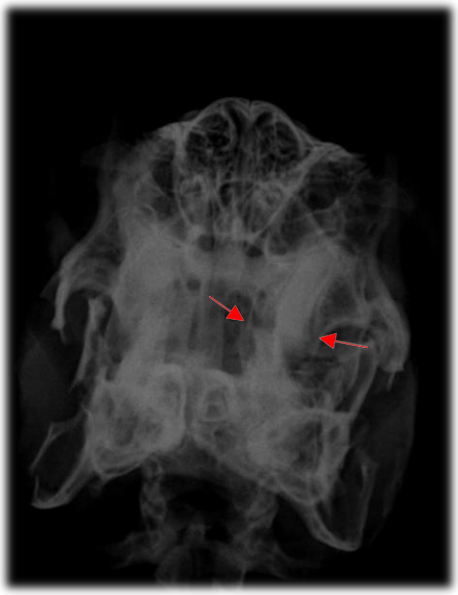
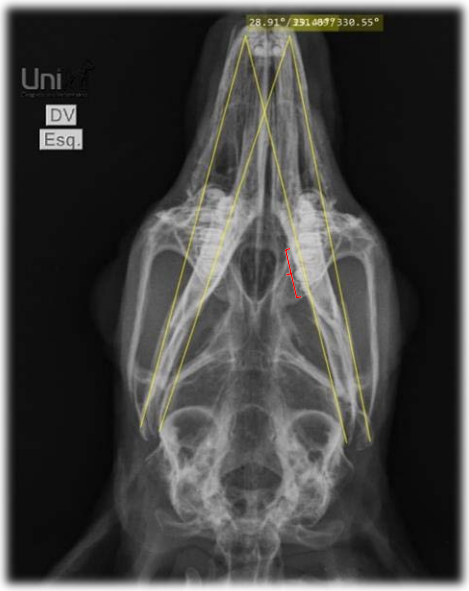
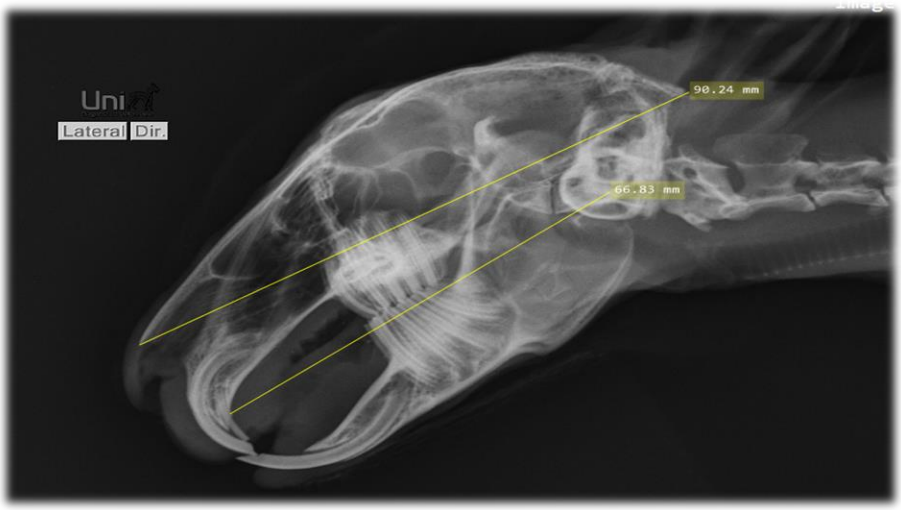


Fig. 8: Radiografia de crânio projeção rostro caudal. Visualização de pontas dentárias evidenciadas pelas setas vermelhas



Fonte: Arquivo pessoal do autor

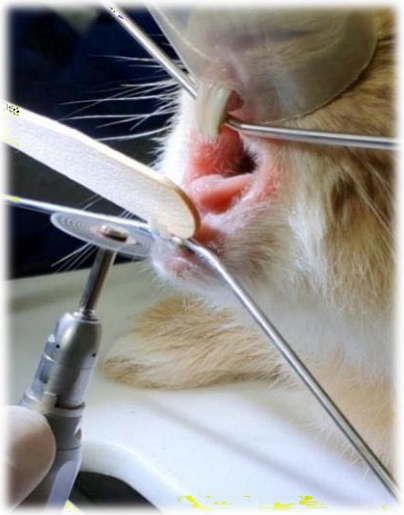
Fig. 9: Radiografia de crânio projeção dorso ventral. Leve desalinhamento dentário na borda medial do ramo mandibular direito, sobrepuljando a linha de referência anatômica amarela, evidenciado em vermelho



Fonte: Arquivo pessoal do autor

Fig. 10: Radiografia de crânio projeção latero lateral visualização de crescimento dentário dos pré molares e molares sobre a mesa oclusal (linha amarela nº 1 – traçada do início do palato duro ao terço inferior da bula timpânica) e também visualização de crescimento da coroa de reserva acima da linha amarela nº 2 que vai do osso nasal ao osso occipital (sugerindo crescimento retrógrado), evidenciado pelas setas vermelhas.

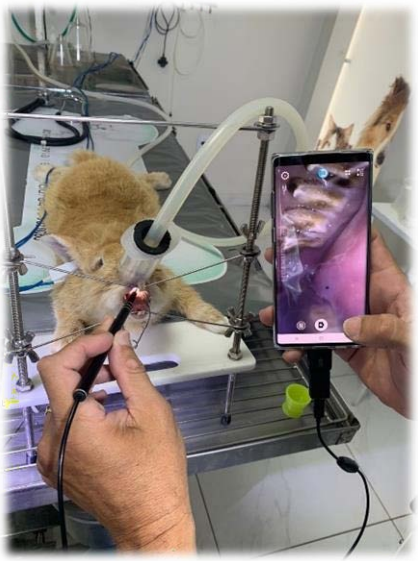
No dia 13 de Setembro de 2021 no Hospital Veterinário Jefferson Ricardo, situado em João Pessoa, Paraíba, o paciente foi operado. O tratamento consistiu na redução da coroa clínica dos incisivos por meio de odontosecção com disco de corte diamantado (Fig. 11 e 12) e dos pré molares e molares usando a peça reta com uma broca carbide esférica nº 8, buscando a oclusão correta dos incisivos, que acontece quando os incisivos inferiores se encontram entre os incisivos superiores anteriores e posteriores e uma angulação próxima dos 10º para os pré molares e molares, que é o preconizado na literatura VESTRAETE, Frank (2005). Essa verificação foi feitapor meio do registro fotográfico utilizando-se uma micro câmera anexada ao celular e também uma câmera digital (Fig. 13, 14 e 15 a,b,c).



Fonte: Arquivo pessoal do autor

Fig. 11: Demarcação do local da ondontosecção com caneta cirúrgica

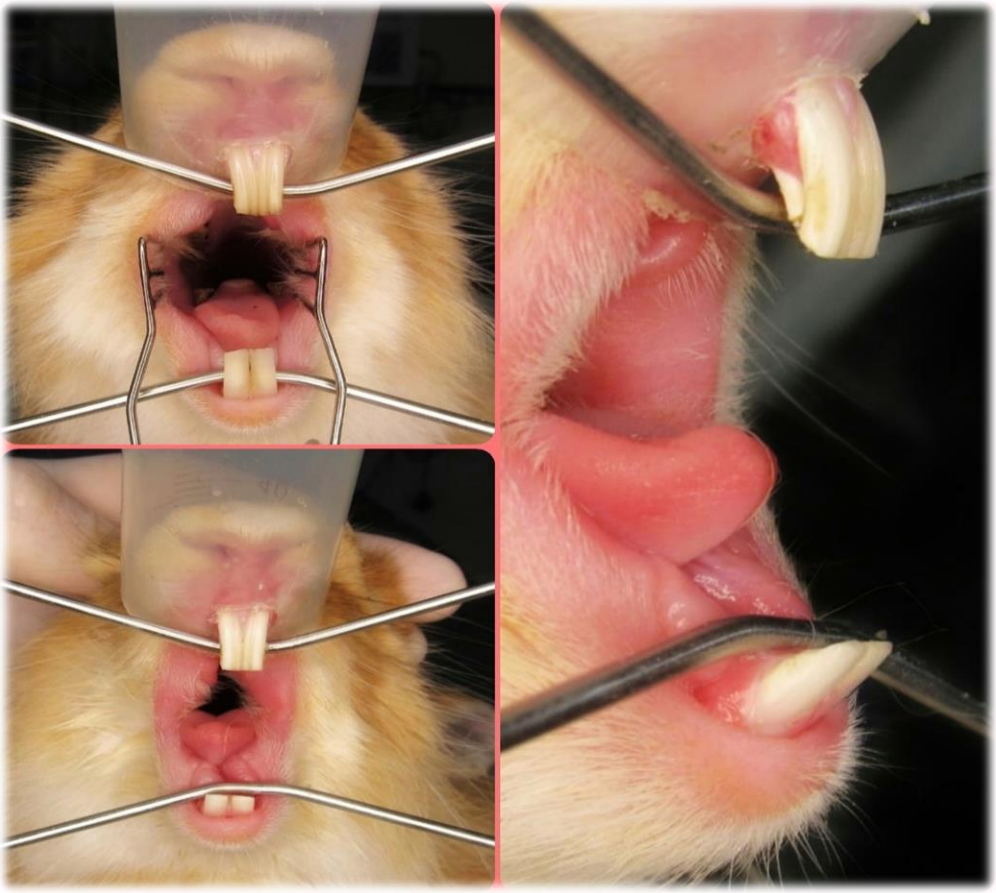
Fig. 12: Odontosecção dos incisivos inferiores



Fonte: Arquivo pessoal do autor

Fig. 13: Uso da Micro câmera para conferência do desgaste oclusal

Fig. 14: Uso da câmera digital para oregistro e documentação do caso



Fonte: Arquivo pessoal do autor

Fig. 15: a. Incisivos aspecto inicial; b. Incisivos aspecto final, vista rostral. c. Incisivos aspecto final vista lateral.

IV. Resultados

O animal retornou bem da anestesia inalatória, deambulando normalmente na baia de recuperação, teve alta e ao chegar em casa prontamente se alimentou de gramíneas fornecidaspela tutora. Com 30 dias voltou para o retorno e o relato era de normalidade, ajuste da dieta eganho de peso.

V. Considerações Finais

Como os coelhos com doenças dentárias geralmente apresentam manifestações clínicas tardias, sinais inespecíficos, de difícil identificação, uma avaliação sistêmica completa antes de iniciar o tratamento odontológico é imprescindível e que pacientes dessa espécie com má oclusão dentária devam sempre receber um exame bucal completo. A pesagem semanal de pets não convencionais mantidos como animais de estimação é fortemente recomendada, pois qualquer perda de peso requer investigação e isso destaca a importância de integrar rotineiramente técnicaseficazes e não invasivas, como a radiografia de crânio, na rotina do exame clínico segundo BOEHMER, Christine (2020). A doença oral pode, assim, ser identificada e o tratamento instituído mais cedo. A dieta ideal para os coelhos, que são estritamente herbívoros, consiste na adoção de uma alimentação rica em fibras para que ocorra o devido desgaste dentário, sendo o capim e feno grosso como componentes principais e podendo ser complementado com vegetais frescos e pellets secos. Se forem fornecidos pellets secos, eles devem formar apenas um máximo de 10% da dieta total, mantendo assim uma boa abrasividade e o nível de desgaste ideal, pois é constante o ritmo de crescimento dos dentes desses animais FERREIRA, Larissa (2020).

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Acknowledgments

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d) An introduction, giving fundamental background objectives.
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g) Suitable statistical data should also be given.
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Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

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j) There should be brief acknowledgments.
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The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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