DEPARTMENT OF EXOTIC ANIMAL AND WILDLIFE MEDICINE,UNIVERSITY OF VETERINARY MEDICINE BUDAPEST

Comparison of the brains of fur chewing and non-fur chewing chinchillas (Chinchilla lanigera) using histology and pathology – A literature review

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1. Introduction

The chinchilla (*Chinchilla lanigera*) is an exotic small mammal, of the rodent family, which is widely used in fur-farms, laboratory research, and increasingly is being kept as an exotic pet.

The topic I am studying is of vast importance, mainly for the profitability of the fur chinchilla trade, but also for the welfare of the chinchillas kept for all three of the above-mentioned reasons.

Initially the aim of carrying out my own research in this project, by performing pathological and histological examinations focused on comparing the brains of Chinchillas affected by fur-chewing to those who are not, was to try and identify a correlating pattern of histological or pathological changes with the behaviour of fur-chewing, to identify a potential pathophysiological cause. Unfortunately, due to the COVID-19 pandemic causing the closure of the university to students, and the closure of Chinchilla Farms, preventing us from gaining more specimens to include in the study, we only managed to complete preliminary practical examinations, therefore I will talk about these preliminary studies, and what following studies I would recommend.

The aim of partaking in an in-depth literature review of related sources on my topic was to find information and summarize the literature available in the surrounding fields, such as:

- Studies of the brain anatomy and histology of chinchillas, giving me a basis to use for comparison in my own research. (AUREL, D., CRISAN, M., 2014).
- Data regarding the commonality of Fur-chewing and comparisons or correlations of the rate of occurrence of fur-chewing and other issues in fur-farmed chinchillas. (FRANCHI, V., ALEUY, A., TADICH, T., 2016).
- Data relating to factors under farm conditions which show correlation with fur-chewing, and attempt to identify patterns which suggest a causative effect. (LAPINSKI, S., LIS, M., WOJCIK, A. Et al, 2014).
- Information regarding Fur-chewing as a stereotypical behaviour, and hence its impact on animal welfare (MASON, G., LATHAM, N., 2004) as well as ways to potentially prevent or reduce incidences of stereotypical behaviours , either by physical methods (MALMKVIST, J., PALME, R., SVENDSEN, P. et al, 2013) or via genetic methods such as breeding out (HANSEN, B., JEPPSEN, L., BERG, P., 2010).

- Studies on the relationship between Nutrition and fur-chewing, is there a correlation between fibre consumption and this behaviour? (BOWDEN, R.S.T., 1962).
- Information about suspected Hormonal or Neural aspects which are involved in the pathological process of Fur-chewing, such as adrenal activity (PONZIO, M., MONFORT, S., BUSSIO, J. et al, 2012) or Thyroid function (VANJONACK, W., JOHNSON, H., 1973) and even Stress-induced Cushing's syndrome (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002).
- Related to the last point I will also be reviewing available data on sampling these animals, such as non-invasive Adrenal sampling (PONZIO, M., MONFORT, S., BUSSO, J., 2004) and using baseline Cortisol levels as an indicator of stress, and potential stereotypies in these animals (SVENDSEN, P., PALME, R., MALMKVIST, J., 2013).
- I also will discuss the relevant available material inspecting the possibilities of treatment for Fur-chewing, such as the use of drugs like Fluoxetine (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013).

1 a) The chinchilla:

The chinchillas kept as fur animals and pets today are descended from hybridising *Chinchilla lanigera* (The long-tailed chinchilla) and *Chinchilla chinchilla (brevicaudata)*. This hybridisation aimed to benefit from the superior fur quality of the *chinchilla*, and the larger litter size and stress tolerance of the *lanigera*.

Basic Information about the chinchilla:

- Life expectancy: 9-16 years
- Weight: 350-650g
- Hair length: 1,8-3,5 cm
- Sexual maturity: 4-5 months
- Heart rate: 100-150 times per minute
- Respiration: 95-170 times per minute
- Body temperature: 37-39 degrees Celsius
- Gestation period: 111 days
- Number of young in a litter: 1–6 (2 is the most usual)
- Number of litters per year: up to 3 litters (1 most usual)

Wild chinchillas are originally from the Andes Mountains of South America throughout Peru, Chile, and Bolivia. They are subjected to considerable variations in temperature, long droughts as well as low humidity. The two species of chinchilla, are both extant, but both the *lanigera* and *chinchilla (brevicaudata* type) are endangered. (THE CHINCHILLA, 2003) The *Chinchilla brevicaudata* has a flatter profile, with a more heavyset appearance, and a shorter tail. *Chinchilla lanigera* has a more triangular profile head, erect ears and longer tail (FIGURE 1)

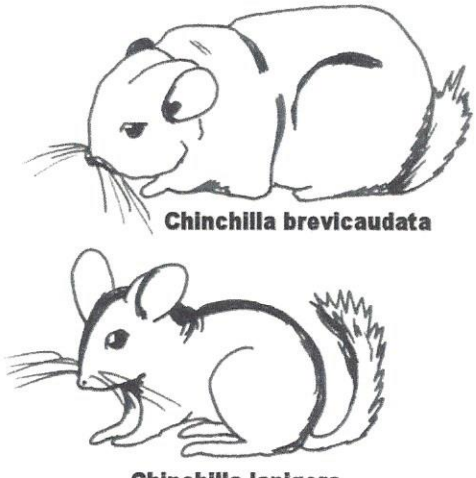

Figure 1: Comparison of *Chinchilla lanigera* and *brevicaudata*. (SALIX, 2006) licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

'The measurements of an adult chinchilla are as follows:

- From the snout to the root of the tail: 28 to 40cm
- Length of the tail: 13 to 16cm
- Length of the ear: 4.5 to 5cm
- Width of the ear: 3 to 3.5cm
- From the ankle to the tip of the nail: 5 to 6cm
- Width of the palm of the hand: 2.5 to 3cm
- Length of the nails of the hand (front paw): 0.2 to 0.25cm
- Length of the nails of the hind paw: 0.35 to 0.40cm
- Length of the whiskers: 10 to 13cm' (THE CHINCHILLA, 2003)

Wild chinchilla eats roots, grasses, grains, bark, fruits, bulbs, leaves, stalks, seeds and nuts. chinchillas, together with the viscachas (lagotis) a separate family called *Chinchillideae*, which, as members of the *Rodentia* order (who, along with the Lagomorphs are counted among the Glires) have teeth which continue growing through the animals entire life and must therefore be worn down to prevent overgrowth and related issues.

1 b) Chinchilla husbandry

Chinchillas are naturally group animals, however in fur-farms they are kept individually or sometimes in polygamous breeding colonies where a system of individual female housing has been devised which allows a single male to serve 12 females (DONNELLY, T., BROWN, C., 2004). Obviously this is not ideal husbandry, although neither is the keeping of singular pet chinchillas. Unfortunately we don't actually know a great deal about the natural behaviour of chinchillas, as it is believed that much of the older literature on wild chinchilla was actually written about *Lagostomus* (plains visacha). In reality chinchilla may be aggressive in group settings, and in the wild may actually be solitary creatures, which is impossible to mimic in captivity. It should also be considered that wild chinchillas hide in burrows and natural rocky crevices, so a hiding place must be provided for captive chinchillas to reduce stress. The environment should also be kept at as constant a temperature as possible '18 to 22°C' as chinchillas are highly sensitive to higher temperatures, though they can survive very low temeratures, and farms keep them between 6-18°C. (DONNELLY, T., BROWN, C., 2004)

Chinchillas nutrition must be taken into account, 'the accepted formula for chinchilla pellets is 16% to 20% protein, 2& to 5% fat, and 15% to 35% bulk fibre' (DONNELLY, T., BROWN, C., 2004), this is important to prevent nutritional disorders, which may be commonly seen when chinchillas are not fed with a pelleted format diet, as they are selective feeders, and are also common where chinchillas are fed diets formulated for other rodent species. It is also important to remember that chinchillas practice coprophagy, 90% of which occurs at night, so a mesh bottomed cage may not be suitable for housing. (DONNELLY, T., BROWN, C., 2004)

There are many other factors to be considered with regards to keeping chinchillas, for one, sexing may be quite difficult, as chinchillas are facultative cryptorchids (DONNELLY, T., BROWN, C., 2004), although with practice a 1-2cm flaccid penis may be extruded from the male and differentiated from the smaller female clitoris. And a majorly important, and unique, factor in fur-farm chinchillas is their requirement for dust bathing, in fact it is noted that 'when denied dust bathing in captivity the fur becomes matted from oily secretions on the back'. (DONNELLY, T., BROWN, C., 2004)

1 c) The Chinchilla Fur Trade

The international trade in chinchilla fur goes back to the 1500's. (THE CHINCHILLA, 2003)

In current times, the fur industry is worth a Great deal of money, as is shown in the table below, in 2015 the total export worth of fur in Europe was €573,412,403. It is worth noting that whilst Chinchillas make up a relatively small fraction of the animals kept in the fur industry in Europe, only 210,000, compared to 39,055,000 Mink for example, they are extremely valuable. (FUR INDUSTRY BY COUNTRY, 2017) (TABLE 1)

Table 1: Table of EU fur production by species and profits (Note: Data for table provided from (FUR INDUSTRY BY COUNTRY, 2017))

		Species				
Country	**Fur Farms**	**Mink (*Neovison vison*)**	**Fox (*Vulpes vulpes*)**	**Finnraccoon (*Nyctereutes procyonoides*)**	**Chinchilla (*Chinchilla lanigera*)**	**Export worth (€)**
Belgium	17	150,000	0	0	0	7,921,498
Bosnia-Herzegovina	50	5000	0	0	22,000	23,384
Bulgaria	2	100,000	0	0	0	1,810,550
Czech Republic	10	20,000	500	0	0	3,582,247
Denmark	1533	17,000,000	2000	0	50,000	8,583,492
Estonia	40	35,000	15,000	0	6500	245,314
Finland	914	1,876,000	2,530,000	152,000	0	1,996,976
France	9	10,000	0	0	0	76,015,679
Germany	3	100,000	0	0	1500	45,287,114
Greece	98	1,600,000	0	0	0	98,908,552
Hungary	213	0	0	0	19,000	1,350,000

Iceland	30	170,000	0	0	0	56,321
Ireland	3	110,000	0	0	0	21,488
Italy	20	180,000	0	0	0	303,732,870
Latvia	8	605,000	5000	0	3000	248,211
Lithuania	131	1,850,000	1500	0	6000	1,084,592
Netherlands	185	4,800,000	0	0	0	4,276,862
Norway	200	590,000	90,000	0	0	189,529
Poland	1144	8,000,000	50,000	8,000	70,000	2,433,699
Romania	153	200,000	0	0	12,000	560,988
Serbia	52	0	0	0	20,000	68,680
Slovakia	1	4000	0	0	0	2,825,574
Spain	54	750,000	0	0	0	9,210,301
Sweden	80	900,000	0	0	0	2,978,482
Total	4950	39,055,000	2,694,000	160,000	210,000	573,412,403

A study in 2018 found that a single chinchilla fur pelt, from a healthy animal was worth, on average, US$38, however, a chinchilla fur pelt from a fur-chewing animal was worth, on average, only US$21.1. This explains why finding the cause and pathological process of this behaviour is so important, as not only the welfare of the animals is affected, but also severe economic losses may be incurred.(GONZALEZ, C., YANEZ, J., TADICH, T., 2018)

In one study an average of 5% of chinchillas surveyed were affected by fur-chewing, if this average number is applied to the total number of chinchillas kept in fur farms throughout Europe, the total number of affected animals becomes enormous. (GONZALEZ, C., YANEZ, J., TADICH, T., 2018)

$$210000 \times 0.05 = 10{,}500\ Fur - chewing\ affected\ Chinchillas$$

$$\$38 - \$21.1 = \$16.9\ less\ per\ affected\ Chinchilla$$

$$\$16.9 \times 10{,}500 = \$177{,}450\ less\ across\ Europe$$

As my above calculations show, the implications of fur-chewing in chinchillas is a very severe economic issue, as well as being a significant issue for vets to face with treating animals with potentially poor welfare, and illness.

2. Literature Review

2 a) Studies of the brain anatomy and histology of the chinchilla (*Chinchilla lanigera*)

The chinchilla is widely used in neurological research, however, there is very little scientific research available regarding the anatomy and histology of the CNS (central nervous system) of the species, which could be an issue regarding the reliability of results of studies using these animals. It is important to note that in lagomorphs and rodents 'the surface of the hemisphere is smooth, except at places where there are deep fissures dividing the hemispheres into lobes' (SAXENA, R., SAXENA, S., 2008) contrasting with higher mammal classes, where the surface is folded into wavy ridges, known as gyri and the depressions between them, called sulci. These increase the surface area of the cerebral cortex without making the hemisphere overly voluminous. (SAXENA, R., SAXENA, S., 2008) This major difference between rodents and other mammals highlights the importance of having studies related to the specific species involved in your research, as even the basic anatomical features may differ drastically.

Some studies carried out research on the anatomy of the Chinchillas brain, comparing its anatomy to that of the closely taxonomically related rat (*Rattus norvegicus*), to see if the Rats anatomy is a viable comparison to draw conclusions from in further studies. A study of the medulla oblongata of *Chinchilla lanigera* found that overall similarities of medulla oblongata neuroanatomic maps of the rat can be utilized as a relevant general guide for the chinchilla. However, also stated that precise neurosurgical experimental models of the species are required for more accurate mapping of the chinchilla's medulla oblongata. (AUREL, D., CRISAN, M. (ed.), 2014a) These results are interesting, as many minor differences in the internal topography of the Medulla Oblongata, such as 'more prominent medial vestibular nuclei, spinal trigeminal tracts and nuclei with a higher position in the anterior half'(AUREL, D., CRISAN, M. (ed.), 2014a) were reported in the study. With regards to the external anatomy, the chinchillas brain is described as several areas tend to be more prominent (ventral pyramids, trapezoid bodies, dorsal vestibular areas), whilst having a narrower rhomboid fossa. The coronal profile of the medulla oblongata of the chinchilla is also proportionally narrower (AUREL, D., CRISAN, M. (ed.), 2014a) Both these internal and external differences suggest that for specific studies of brain changes, such as in my thesis research, a control sample of chinchilla brains from unaffected animals should be used, rather than relying on reports of normal brain anatomy and histology from other rodent species. This study was very clear in its explanation, and described the method simply, and seems reliable as a representative sample of real farm kept chinchillas was used for the sampling, including ten encephalon's sampled from commercially slaughtered adult chinchillas of both sexes. (AUREL, D., CRISAN, M. (ed.), 2014a) Of these samples, five were used macroscopically to study the anatomy and 5 were examined histologically, which gives a good overview of differences to be taken into account both externally and internally between Chinchillas and other Rodent species.(AUREL, D., CRISAN, M. (ed.), 2014a)

One study compared the cerebral hemispheres of *Chinchilla lanigera* to both rats and rabbits (*Oryctolagus cuniculus*), which provides two interesting comparative study points, as rabbits are of the lagomorph order, whilst rats and chinchillas are both under the rodent order, however all of these species are Glires. This study also was based around the fact that the CNS system of chinchillas is vastly ignored by scientific study, whilst the species is widely used in studies regarding neuroanatomy. In this study only five brains were studied, however the animals used were from commercially slaughtered chinchillas of both sexes, so the sample was representative of the overall population, and the brains were only studied macroscopically, so didn't provide detailed information on differences between species brains down to a histological level. The results of this study show that 'The surface of the cerebral hemispheres in the chinchilla shares the lissencephalic characteristics of rodents and rabbits.' (AUREL, D., CRISAN, M. (ed.), 2014b), lissencephaly being a smooth outer surface of the brain (lack of sulci and gyri). It is also noted that a ventral view of the brain reveals a well-developed rhinencephalon similar to a rat's however has a similarly narrow shape to the rabbit. And 'The pyriform lobes have a particular globular shape.' (AUREL, D., CRISAN, M. (ed.), 2014b) Whilst the dorsal view of the cortex shows that the oral halves of the hemispheres are significantly contracted, which creates a triangular shape which is seen similarly in the rabbit. The study also found that the general frame of the hemispheres was more compact and the ventral profile was less curved which bears more resemblance to that of a rat. (AUREL, D., CRISAN, M. (ed.), 2014b) This set of results reflects in the conclusion of the study, which bears the opinion that further studies should be carried out on the anatomy of the chinchillas cerebral hemispheres, as the brain of the chinchilla is not represented well by the normal descriptions of any other single species. (AUREL, D., CRISAN, M. (ed.), 2014b)

Another study looks at the macroscopical and histological aspects of the cerebellum of the chinchilla, where, as a laboratory animal, their main use consists of experimental models in hearing pathology and auditory pathways research which is due to several peculiarities of the species, which include a three-chambered tympanic bulla and a very similar cochlear structure to that of a human along with a high resistance to ear infections. (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) However also acknowledged, just as the above discussed papers, that there is a vast amount of work required to catch up with the knowledge already gathered on the neuroanatomy of other lab animal species such as the rat. As with other studies of this ilk, the sample for the study consisted of five samples prepared histologically and five samples observed macroscopically, collected from chinchillas of both sexes, of adult age, giving a proper representative study of the entire population (though not accounting for development and changes with age). According to this study, the chinchilla has several differences to other mammalian brains reported in literature, for example its global shape, relations to the cerebral hemispheres and colliculi as well as the hemispheres/ vermis development ratio (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) which are expectable when considering the species neurocranium. It is also notable that the general shape of the cerebellum in chinchillas is rather different when compared to that of the rat and that of the rabbit. Chinchillas are described as having a globulous aspect, while rabbits and rats featured a more cylindrical shape, because they are flattened rostro-aborally. (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) Not all of the data from this study suggests that the chinchilla cannot be compared to other species for reference data however, as some aspects of the neuroanatomy appear very similar to related species, such as the relation between the cerebellum and the cerebral hemispheres which was found to be similar in all three species, and none of them have the aboral segment of the telencephalon covering the cerebellum. (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) The study states that its histological date regarding the 'three lamellar structure of the cerebellar cortex'(IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) is in line with the general mammal and rodent guidelines, mentioning 'granule cells of the granular layer, which measured approximatively 5 micrometres in the chinchilla samples, subscribe to the average of 5 to 8 micrometres'(IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) however, also states that 'the size of the Purkinje cells in chinchillas averaged 15 by 20 micrometres, which is a lot smaller than the average measurements of 50-80 by 30-40 micrometres'(IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) and it is also mentioned that the 'mass of cerebellar nuclei compared to its descriptions in literature' 'also indicate some particularities, as its aboral region is larger than that in the rat.' (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015) It should also be noted, that within the study itself it is mentioned that the histological data, whilst sufficient for use as a guideline, has not been subjected to a statistical analysis. The study concludes in a similar fashion to other literature in this field, stating that there is a need for further studies in this field, because whilst a good basis, the rat neurological model is not an accurate portrayal of some of the peculiarities of the chinchillas neuroanatomy. (IRIMESCU, I., BOLFA, P., CRISAN, M. et al, 2015)

2 b) Studies Observing Fur-chewing as a Stereotypical (Behavioural) issue, and its use as a Welfare marker in Fur-Farming

Stereotypies or stereotypical behaviours are repetitive, unvarying behaviour patterns with an unknown reason (MASON, G., LATHAM, N., 2004) and are frequently associated with poor levels of animal welfare. It is the goal of many studies and programmes to reduce the instance of stereotypical behaviour, such as in zoos where it is one of the most common aims of environmental enrichment programmes for the captive animals and it is also worth noting that in many captive populations stereotypies have been genetically selected against, for example in hens and mink. (MASON, G., LATHAM, N., 2004) However there is relatively little definitive information available with regards to how important reducing stereotypies is for the animals welfare, and whether sometimes the method for reducing the rate of stereotypical behaviour may lower the welfare further, such as using greasy or hot tasting materials to reduce crib-biting in horses. It is also questionable whether enrichment has a negative correlation with stereotypical behaviour, and in some cases certainly does not, as mentioned in this paper, 'providing mink with playballs also increases their stereotypies' and 'stereotypy increases with increasing cage size in artic foxes'. (MASON, G., LATHAM, N., 2004) It should also be noted that some stereotypical behaviours may be referred to with different terminology, such as do-it-yourself enrichments such as grazing-like tongue-playing in calves, which has been associated with reduced levels of gastric ulcers in cattle. (MASON, G., LATHAM, N., 2004) This suggests that we cannot use a blanket statement that stereotypies incidence level will reflect the level of animal welfare, but it should be kept in mind that some stereotypical behaviours, whilst not necessarily indicating a poor level of animal welfare, may actually cause a decrease in the level of animal welfare. (MASON, G., LATHAM, N., 2004) It is also worth considering that rodents (along with primates) have more complex stereotypies than other taxa (such as carnivores or ruminants), comprising of oral and locomotor stereotypies alongside other (often idiosyncratic) motor patterns. (MASON, G., RUSHEN, J., 2006) This may suggest that they are more indicative of poor welfare, as they are a complex behavioural anomaly, caused by a frustrated behaviour.

The behaviour and therefore the enrichment of rodent species are complex issues and therefore enrichment is an important part of maintaining good animal welfare standards and in the case of the fur-industry, ensuring decent profit margins. (BRANDAO, J., MAYER, J., 2011) Examples of a rodent stereotypy include fur-chewing in chinchillas, barbering in mice and compulsive digging in gerbils. It is mentioned in this study that a 'critical factor for chinchillas'(BRANDAO, J., MAYER, J., 2011) is a shelter for better regulation of temperature and humidity, however, in fur-farming this is already controlled within the farm conditions. Chinchillas also require frequent dust baths between once a day to several times a week for coat health, which is also provided in farms, via a scoop of marble dust on the cage floor, this is especially important to fur-farmers as it maintains coat health, removing excess oils and moisture. (BRANDAO, J., MAYER, J., 2011)

Several studies involving mink focused on stereotypic behaviour and how to reduce it. One of these studies focused on reducing the instance of stereotypical behaviours via selection, and concluded that the method was successful in achieving this goal, whilst also having the negative side effect of lowering average litter size. This study is explored in depth in the chapter of this literature review exploring the treatment of fur-chewing. (HANSEN, B., JEPPSEN, L., BERG, P., 2010) The second study looks at using additional foraging materials to reduce the instance of fur-chewing and other stereotypies in fur-farm mink, and describes fur chewing as resembling eating or grooming behaviour, and therefore hypothesizes that by improving the chance of actually exhibiting such behaviours in a non-stereotypical way (in this instance by increasing the particle size of the feed, to improve the eating behaviour) then the instance of stereotypies should decrease, improving the welfare standards of the affected animal, as they display more natural behaviour. (MALMKVIST, J., PALME, R., SVENDSEN, P., et al, 2013) The females in this study showed a reduction in fur-chewing behaviour when fed with 'chunkier' food (36.2% of females carrying out the behaviour down to 24.5%, and the severity also decreased from 0.9+/-0.18 to 0.41+/-0.12) this suggests that the provision of a more natural behavioural trait reduced the incidence of stereotypical behaviour, and therefore also increased the welfare standards of the affected animals. (MALMKVIST, J., PALME, R., SVENDSEN, P., et al, 2013) In this trial, faecal cortisol metabolites (FCM) were also measured, cortisol is the hormone most commonly used as a measurement of stress in animals, and therefore may be used as an indirect measure of animal welfare by means of showing the stress levels they are experiencing. In this case the female mink fed the 'chunkier' diet had a higher concentration of FCM (402+/-30.4ng/g) when compared to the females fed the original commercial diet FCM= 298+/-27.9ng/g, this is a very unusual result, as it does not match with the expected results as seen in the incidence rates of fur-chewing observed, however, the authors of the study also comment that this may be an anomaly in the data due to the weight loss of the mink in the 'pre-mating period;

cortisol being involved in energy mobilisation'. (MALMKVIST, J., PALME, R., SVENDSEN, P., et al, 2013) Further studies mentioning hormonal relationships with fur-chewing, specifically in chinchillas are discussed in a further specific chapter of this literature review.

Some studies don't refer to fur-chewing as a stereotypy, but instead refer to it as an abnormal repetitive behaviour (ARB), and according to this study 'it has been established that 68% of the situations that favour the development of ARBs are also causal factors of poor welfare' (FRANCHI, V., ALEUY, A., TADICH, T., 2016) and also states that they can be used as welfare indicators. It is also a point for consideration that the conditions of the housing systems are considered as important risk factors for a multitude of welfare problems. And with this in mind, it has been established that solitary housing has been associated with an increased risk of ARB in other fur-farming species including fox and mink (FRANCHI, V., ALEUY, A., TADICH, T., 2016), and therefore should be considered for the welfare implications of keeping chinchillas in single-housing, as they are currently in fur-farms, as this likely increases the chances of fur-chewing. It is also interesting that the Welfur Project uses ARBs and fur-chewing as a welfare measure in foxes and mink, yet currently do not have a welfare measure for Chinchillas. (FRANCHI, V., ALEUY, A., TADICH, T., 2016).

2 c) Research observing the relationship of Nutrition and Nutritional issues to Fur-chewing

The chinchilla is a member of the South American infraorder of Caviomorpha which consists of the chinchillas and guinea pigs who are described as folivorous herbivores with their diet consisting of various roots, leaves, fruit, berries, barks, alfalfa, grasses, shrubs and cacti, changing in composition throughout the year and range. This diet is naturally extremely high in fibre, making up >66% of their diet. (GRANT, K., 2014) And it is therefore recommended that to maintain a natural as possible diet, in captivity chinchilla diets should be based on grass hay.

Table 2 Nutrient requirement for chinchilla, guinea pig, hamster, and gerbil (GRANT, K., 2014)

	Chinchilla40,42	Guinea Pig23,25	Hamster28,30,41–44	Gerbil29,31,43–46
Crude protein (%)	16–20	10–16 (adult) 18–20 (growth)	16–20	16 (adult) 17–25 (growth)
Fat (%)	2–5	NA	4–5	2–5
Fibre (%)	15–35	>15	>15	6–10
Calcium (%)	0.60	0.80	0.60	0.50
Phosphorus (%)	0.40	0.40	0.35	0.30
Vitamin A (IU/kg)	NA	6.6	1.1	0.7
Vitamin C (mg/kg)	NA	200	NA	NA

With regards to fur-chewing, it is worth noting that this study refers to the fact that a crude protein deficiency may be a cause of a poor hair coat, which could be a differential diagnosis for fur-chewing. It also discusses the fact that 'Coarse hay (alfalfa, orchard grass, timothy, blue grasses) with 14% to 16% crude protein prevents tooth overgrowth', (GRANT, K., 2014) which is of importance, as traditionally dental issues have been linked to fibre deficiency and then to fur-chewing, as the fur is supposedly chewed as a replacement for the missing fibre, and chinchilla have a much higher fibre requirement than even other rodents (TABLE 2). It is also mentioned that fur-chewing may lead to further pathologies such as 'Gastric trichobezoars: result of fur chewing and lack of dietary fibre' (GRANT, K., 2014) which may be a useful tool for detection of these issues in gross pathological studies of chinchilla corpses. Another study looked at the diet of wild *Chinchilla lanigera* via monitoring their fresh faeces, based on research showing that plant species composition in the diet varied considerably among the year's seasons. The study was rather reliable as more than 100 faecal pellets were collected in a rainy season and a dry season, and 40 of each set of these were randomly selected and underwent dietary analysis. The results showed that whilst the diet varied tremendously by season (wet or dry), 'overall, fibres made up most (>66%) of the diet in both years and in all seasons' (CORTES, A., MIRANDA, E., JIMENEZ, J., 2002) which suggests that this is the most important factor of chinchillas diet, and should therefore be taken into account in captive chinchilla keeping also. Another interesting finding from the study was that when offered both dead and live fresh leaves of the same plants, chinchillas most often preferred to consume the dead leaves which would also suggest that high fibre levels are preferable and even selected for by chinchillas. The vast variety of foods ingested by chinchillas in the wild 'up to 24 plant species, mainly herbs'(CORTES, A., MIRANDA, E., JIMENEZ, J., 2002) is not replicated effectively at all in captivity, and could therefore contribute to a lack of stimulation, which is purposed to cause increased levels of stereotypic behaviours (such as fur-chewing) in captive animals.

A study on dental disease on chinchillas performed examinations on chinchillas from various backgrounds (pets, rescue centres and breeding colonies) who were brought for presentation for various reasons, and found that suspected malocclusion or dental disease was responsible for 68%, fur-chewing for 21% and other signs for 11%, the reason for culling these chinchillas for examination was given in the study as fur-chewing is seen as 'breeding problems or signs believed to indicate impending or existing dental disease' (CROSSLEY, D. A., 2001) Another gross post-mortem exam carried out on 104 chinchillas in this study found 39% were affected by fur-chewing. Unfortunately in this study no data was provided about whether the signs of fur-chewing did correspond with the signs of dental problems, so in reality the data cannot be relied on, other than to highlight the fact that an enormous (up to

39%) of the chinchillas culled in fur-breeding, are culled due to fur-chewing. (CROSSLEY, D. A., 2001)

One study looked at the effect of feeding pelleted diets and hay roughages on instance of fur-chewing. This study was quite limited, analysing only fifty-eight animals, and may not be very reliable, as it used animals from a variety of backgrounds, some of which were fur-chewers and some of whom were not at the start of the trial. It is stated that the purpose of the experiment was to ascertain whether withholding hay over long periods of time would have any influence on the incidence of fur-chewing in the population and conclusively proved that it did not. Additionally the conclusion of this study noted that 'no fur-chewer was "cured" whether it had the habit on arrival or developed it after', (BOWDEN, R.S.T., 1962) which suggests that nutritional treatment alone has neither preventative of treatment effects on fur-chewing. Further studies would be interesting if they used a standard group of chinchillas, raised from weaning using groups fed on different pellets and roughage combinations, which were divided into fur-chewers and non-fur-chewers to carry out a fair and well analysable test.

2 d) Research observing the relationship of hormones and their disorders to Fur-chewing

One study looks at adrenal activity and anxiety-like behaviour in chinchillas that fur-chew, hypothesizing that increased levels of this abnormal behaviour would show correlation with higher levels of adrenal activation. The chinchillas were kept within environmental and management conditions which were the same as those utilised in commercial breeding farms around the world. This shows that the environmental impacts on the results were minimized. Two experiments were carried out in this study, one to measure the cortisol metabolite excretion, and one to measure anxiety-like-behaviour, using the elevated plus maze test (EPM). In this study females whom exhibited the most severe form of fur-chewing also excreted significantly elevated concentrations of cortisol within the urine which would suggest that stress is at least involved in the mediation of the behaviour. However, it is also noted by the authors that there was no relationship between the increasing severity of the fur-chewing behaviour and the level of cortisol metabolites in the excretion. This leaves the area open to further testing to establish more data on the relationship of stress and fur-chewing, and to answer why it was that only severely fur-chewing female chinchillas showed an increase in cortisol excretion. (PONZIO, M., MONFORT, S., BUSSIO, J. et al, 2012)

Table 3 Urinary cortisol metabolites concentration in domestic *Chinchilla lanigera* showing different levels of fur-chewing behaviour. (PONZIO, M., MONFORT, S., BUSSIO, J. et al, 2012)

	Cortisol metabolites (μg/mg creatinine)				
	Normal	Slight	Moderate	Severe	Very severe
Females	635.6±66.9 (8) a	493.5±114.9 (4) a	682.4±195.6 (5) a	969.2±188.7 (6) a	1652.6±623.8 (5) b
Males	981.8±172.2 (6) a	594.2±83.9 (5) a	1156.0±155.4 (6) a	619.4±80.4 (5) a	904.3±200.6 (9) a

'Data are expressed as the mean of the medians obtained for each group±SEM. In parenthesis, number of animals in each group. a vs b (in rows): P=0.04.' (PONZIO, M., MONFORT, S., BUSSIO, J. et al, 2012)

The general trend of results shows correlation between the severity of fur-chewing and the levels of cortisol metabolites, however is much clearer in females, and becomes most apparent in very severely affected females. (TABLE 3) The EPM test also showed an increase in anxiety related behaviours, for example, a decrease in the percentage of entries and time spent in open arms, and increasing rates of freezing behaviour, once again only in the fur-chewing females. (PONZIO, M., MONFORT, S., BUSSIO, J. et al, 2012)

The same authors of the study on the adrenal activity of chinchillas in fur-chewing also authored a paper on non-invasive methods of sample collection, for adrenal measurements. The traditional method for this was via the evaluation of serum and plasma glucocorticoid concentrations, however due to their very small vein size and their highly stress-susceptible nature this was a difficult and unreliable source of data, therefore a more modern approach of using non-invasive methods such as faecal and urinary corticosteroid concentration monitoring is employed to avoid causing unnecessary stress. (PONZIO, M., MONFORT, S., BUSSO, J., 2004)

Table 4 Excretory fate of injected ^{3}H-Corticosterone in *Chinchilla lanigera* (PONZIO, M., MONFORT, S., BUSSO, J., 2004)

	% Total radioactivity excreted	% Water soluble forms	% Ether soluble forms
Urine	86.9 +/- 0.07	90.5 +/- 5.7	9.5 +/- 5.7
Faeces	13.1 +/- 0.08	20.8 +/- 14.3	79.2 +/- 14.3

'The values are expressed as mean +/- SEM.' (PONZIO, M., MONFORT, S., BUSSO, J., 2004)

Most of the experimental corticosterone given to the chinchillas was excreted in urine (86.9+/-0.07), which is useful information for further studies involving the measurement of such hormonal products in chinchillas specifically, as it is now known, that urine is an effective source for monitoring these products effectively. (TABLE 4) (PONZIO, M., MONFORT, S., BUSSO, J., 2004)

Whilst discussing sampling of chinchillas, it is also worth noting that to ensure that the issue being dealt with is indeed fur-chewing and not another pathological process with similar symptomatic appearance a skin scrape or fur sample culture may be used. For example ringworm caused by Trichophyton mentagrophytes is quite common in chinchillas. The usual pattern of ringworm will include small scaly areas of skin with alopecia, most commonly on the nose, behind the ears, or on the forefeet, although lesions may develop anywhere on the body. That could possibly be confused with severe fur-chewing in untrained persons. (NESS, R., 1999)

Some studies have been carried out also in mink (*Neovison vison*) another fur-farm kept animal, as a novel study relating the severity of fur-chewing to the baseline cortisol levels of the animals, to try and establish whether levels of stress directly correlate with the levels of fur-chewing. This, according to the report, is important as fur-chewing is commonly used as an indicator of lower welfare levels in mink and although not officially used as such in chinchillas, it is fair to assume that the same behaviours in different species kept on similar farms/under similar conditions can be used in the same fashion. The study was relatively large scale, using 200 mink under identical conditions which is a good basis for collecting reliable data, however, all of the mink were female, which limits the use of the data set in providing information over the whole fur-farm mink population, as no data was gathered regarding male Mink. In this study, the cortisol measurements were taken non-invasively, however in contrast to the studies in chinchilla showing that the best method for measuring corticosteroid excretion being urine, this author stated that 'The main excretory route of cortisol metabolites in mink is via faeces (83% reported for females)' (SVENDSEN, P., PALME, R., MALMKVIST, J., 2013), so Faecal cortisol Metabolites (FCM) were used.

This study found clear links between FCM and tail-chewing (part of the stereotypical behaviour). FCM was found to be extremely high in levels in tail-chewing mink that carried out 5 and above repetitions of stereotypic behaviour in observation. It was also observed that tail-chewing mink generally had higher baseline cortisol levels (FCM) compared to those minks who did not tail-chew, which of course suggests that tail-chewing mink may be interpreted as more stress-sensitive animals considering their HPA-axis (hypothalamic-pituitary-adrenal axis) output (SVENDSEN, P., PALME, R., MALMKVIST, J., 2013), which interestingly reflects the findings of the study I previously described carried out in chinchilla (PONZIO, M., MONFORT, S., BUSSIO, J., et al, 2012).

A study of twelve chinchillas (six normal and six fur-chewers) was carried out in an attempt to prove a relationship of fur-chewing to thyroid and adrenal functions. Obviously this is a fairly small sample size, especially when considering measurements were only taken at 12 and 18 months of age, although it must be stated that management conditions were standardized for all participating chinchillas, and were uniform for the duration of the entire study. (VANJONACK, W., JOHNSON, H., 1973)

It was found at the 12 month measurement using 'thyroid mI release rates (k) as a measure of thyroid activity, fur- chewers had a significantly higher (P< 0.001) k value (8.44) than normal controls (5.73)' (VANJONACK, W., JOHNSON, H., 1973) which was further backed up when the fur-chewers' k value was significantly higher than the control at 18 months of age, showing a clear correlation between increased thyroid activity and fur-chewing. This evidence was further confirmed when 'A significantly higher TSR (3.07/~g L-T4/100 g (b.w. per 24 hr) was obtained in the fur-chewers (compared to a normal value of 2.35).' (VANJONACK, W., JOHNSON, H., 1973) TSR being thyroid secretion rate. Also within this study we can see histological samples of the chinchilla's thyroids, in which we see 'The fur-chewed group appears to have higher columnar cells of the secretory epithelium and less colloid' (VANJONACK, W., JOHNSON, H., 1973) which would support the above data, that the gland is more active in its formation.

Once the study has dealt with the data regarding the thyroid gland it moves on to a second investigation into the adrenal activity of the chinchillas, this showed that 'Fur-chewed animals had significantly higher (P< 0.001) corticosterone plasma levels (0.451/~g/ml compared to 0.253 μg/ml for the normal)' (VANJONACK, W., JOHNSON, H., 1973) suggesting an increased adrenal activity in these animals, this theory was supported by histological evidence, within which the fur-chewed group displayed an excessive adrenocortical hypertrophy. Which, when considered together is conclusive evidence of an increased adrenocortical state of function. (VANJONACK, W., JOHNSON, H., 1973)

In this study, the rectal temperature was also measured, which showed means of '35.96°C at 12 months of age and 35.92°C at 18 months of age. At both ages, they were significantly lower (P< 0.01) than the normal controls (36.75 and 36.46°C, respectively).' (VANJONACK, W., JOHNSON, H., 1973) The authors questioned whether this was a consequence of the hormonal changes or the fur-chewing itself, and therefore calculated fur loss, showing 'The fur-chewing group had 50 per cent less fur (weight/area) over their chewed areas, which would account for the less insulation' (VANJONACK, W., JOHNSON, H., 1973) however it is also mentioned that 'the decreased body temperature acting directly on the central nervous system could provide a deep body as well as a peripheral skin stimulus via the hypothalamus (CRF, TRF) and anterior pituitary (ACTH, TSH) for increased thyroid and adrenocortical activity' (VANJONACK, W., JOHNSON, H., 1973). This query is unanswerable with the provided data, and therefore is an interesting question in the pathogenesis of fur-chewing, and should be considered for further research. Overall the study concludes that fur-chewing chinchillas show an increased level of thyroid activity, alongside elevated plasma corticosterone levels with a decreased rectal temperature compared to the control chinchillas. Although it should also be taken into account that the corticosterone levels are measured in an intrusive manner, possibly causing aberrant data due to stress, and the data does not provide enough information to establish a pathogenesis for the reduction in rectal temperature in fur-chewing chinchillas. (VANJONACK, W., JOHNSON, H., 1973)

A further research paper using eleven fur-chewing chinchillas, as well as three healthy control animals, looked at a wide variety of examinations in an attempt to identify a pathogenesis, they carried out pathomorphological exams, bacteriological and mycological studies and parasitological studies. The gross pathological report showed that 'The chewed hair was not present in the gastrointestinal tract. While adrenal hyperplasia was confirmed in all fur-chewing chinchillas' (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002) and in the normal animals, no gross lesions were observed. No pathological bacteria or parasites were discovered, and no dermatophytes, just Mucor species in two of the skin samples. (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002)

Meanwhile, the histopathological examination yielded more interesting and potentially useful results. Stating that the adrenal glands showed a diffuse cortical hyperplasia on the whole, which reflects the gross adrenal hyperplasia recorded in the necropsy. 'The epidermis was hyper keratinised (orthokeratotic or anuclear hyperkeratosis), which was associated with simultaneous epithelial atrophy (1–2 cell thickness) as well as a pronounced follicular and sebaceous gland atrophy' (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002) which, although a symptom of many atrophic follicular skin diseases, when combined with the following 'some cases a telogen-type atrophy of the hair follicles was present. Most of the thin-walled follicles contained keratin material (comedo formations)' (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002), is particularly interesting as comedo formations in thin-walled follicles is usually only observed in Cushing's syndrome. This is an interesting finding, especially when considering that some behavioural changes seen in canine hyperadrenocorticism, namely self-mutilation and aggressiveness surmise fur-chewing in chinchilla very effectively. Undoubtedly these findings pave the way for further more in depth studies, especially to further study the correlation of stress-susceptibility and these Cushing's lesions in chinchillas. (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002)

2 e) Studies of the prevalence of Fur-chewing and Correlation of various husbandry factors with Fur-chewing

As described in other chapters of this literature review, there are a great many theories describing the cause of fur-chewing, and it even appears that there is great variance in the estimated prevalence, 'incidence of fur chewing in farm-raised chinchillas has been shown to range from 4% to 30%' (TYNES, V., 2013) This activity is also known as barbering, and the pathogenesis has been attributed to various factors including behaviour, husbandry, nutritional, metabolic and endocrine factors and it is also noted in some literature that fur-chewing has genetic roots and predominates in certain family lines, in other words, there is a theoretical heritability, and as such breeders frequently remove fur-chewing chinchillas from their breeding colony. (ELLIS, C., MORI, M., 2001) It should also be noted that environmental factors appear to play a large role, and some literature states the importance of 'Room temperature should be 16° to 21 °C (60°-70°F), and relative humidity should be maintained at 40% to 60%.' (ELLIS, C., MORI, M., 2001), although, this clearly is not a direct causative link, as there must be a predisposing factor in these 4 to 30% of affected chinchillas, otherwise whole populations would be affected within farms, and no reports of fur-chewing in wild *Chinchilla lanigera* exist, where the humidity and temperature are not so carefully maintained.

A study in mice showed similar results of barbering (Whisker-trimming) as studies in fur-chewing in chinchillas, and was also compared to trichotillomania (hair-plucking) in humans. This was a large scale study, incorporating 2950 mice of varying ages and strains. The fur lesions were scored as barbering if: '(1) the lesion was non-pruritic (that is the exposed skin was neither reddened nor inflamed), (2) there was no scarring or scabbing around the lesion, (3) the animal was otherwise in good health and the fur where present was in good condition, (4) no other cause for the lesion could be ascertained.' (GARNER, J., DUFOUR, B., GREGG, L. et al, 2004). The severity of barbering and the number of barberers per cage were calculated using a mouse-map and a database, and then a process of elimination for multiple barberers. The results of this study showed that only two husbandry-related risk factors showed significant correlation with cagemate-barbering; these were the cagemate relationship and the cage material. It was shown that 'mice in steel cages were 1.82 times as likely to barber as mice housed in plastic cages.' (GARNER, J., DUFOUR, B., GREGG, L. et al, 2004) And it was also shown that 'Mice housed entirely with siblings were 3.66 times more likely to barber than mice housed entirely with non-siblings' (GARNER, J., DUFOUR, B., GREGG, L. et al, 2004) This study was interesting theoretically when applied to chinchillas, as the behaviour pattern is very similar, and the husbandry-related issues clearly took impact upon the prevalence rate of barbering (something vastly understudied in chinchillas) however, with no direct study carried out on chinchillas, the information is not directly applicable, especially due to the fact that the majority of chinchillas in fur-farms are kept in single keeping cages.

One study specifically looked to analyse factors increasing the probability of chinchilla suffering from fur-chewing in fur-farms, using data gathered in anonymous surveys at fur-farms in Poland, this is excellent from the point of view that the chinchilla were being observed as naturally as possible, with no new people or stressors, however the results are less scientifically acceptable, as they were provided by untrained, and frankly biased sources (the farmers themselves) who may be worried about perceptions of animal welfare on their farm, and quality of fur they produce. The results of these questionnaires showed that 'FCP were found in most farms (85%, $P \leq 0.05$). However, FCP usually concerned a few animals in the herd (mean ± SE: 3.5±0.55%, range 0–15%)' (LAPINSKI, S., LIS, M., WOJCIK, A. et al, 2014) (FCP being Fur chewing problems). These values appear to roughly fit into the range from other studies which say that the incidence of fur-chewing in farm-raised chinchillas has been shown to range between 4% and 30% (TYNES, V., 2013) and a slightly lower than true value should be expected due to the self-reported answers from the farm owners. It was also reported by the farmers via the questionnaires that they '(55%) tried to prevent FCP by changing the feeding (35.5%) and less often by culling (12.5%) or transferring animals to other cages (7.5%)' (LAPINSKI, S., LIS, M., WOJCIK, A. et al, 2014) which is interesting, as the pathogenesis is currently unknown for fur-chewing, but most recent studies suggest a stress based Cushing's syndrome type disorder could be the causative agent (TILJSAR, M., JANIC, D., GRABARENIC, Z. et al, 2002) however, because no effective treatment plan has been studied and published, the farmers still use the old methods they have always used, assuming the cause of the fur-chewing to be either nutritional, or genetic, even with no definitive evidence, or evidence that their treatment methods are effective. Another interesting point raised, which may now be viewed as a husbandry-related risk factor increasing prevalence of fur-chewing in chinchillas is the difference observed in instance due to cage/substrate type provided for the animals. The results of this study showed 'fur-biting animals kept on deep litter was estimated at 1.7±0.49% while on the wire floor and in the mixed system it was 2.8-times higher' (LAPINSKI, S., LIS, M., WOJCIK, A. et al, 2014) which implies that even though not an effective treatment, the risk factor of fur-chewing may be lowered by keeping chinchillas on deep litter. This study however, did not find any relationships between fur-chewing and season, temperature, humidity, type of bathing basin and dust, or frequency of dust bathing per week, it was also reported that no significant relationship was observed between prevalence of fur-chewing, and cage storey. (LAPINSKI, S., LIS, M., WOJCIK, A. et al, 2014)

2 f) Studies of possible Treatments for fur-chewing and their effectiveness

There is relatively little literature to be found regarding the treatment of fur-chewing in chinchillas (and other fur-farmed species); however within the available studies there are a variety of approaches to treating the problem, ranging from drugs, to genetics, to behavioural distractions.

One study looks at reducing fur-chewing in mink using foraging elements, as a behavioural distraction. This study was based on the theory that hunting and eating in predatory animals are completely separate instincts, which will both require satisfaction independently from each other. Rather than the alternate theory that home range size is a cause of stereotypic behaviours. Evidently this study is not necessarily directly applicable to chinchillas, as it is referring to carnivorous animals, and chinchillas are herbivorous, however it is worth noting that appetitive and consummatory foraging behaviour in herbivorous animals may also explain the types of stereotypic behaviour, which is similar to those of predatory animals. (MALMKVIST, J., PALME, R., SVENDSEN, P. et al, 2013) These have been seen in various species of farmed animals (herbivorous species) e.g. feather-pecking in chickens. This was a rather large scale of two hundred mink, managed to commercial standards of European legislation, so can be seen as a relatively reliable study. This was a bi-factorial study, as 'appetitive' (with a rope for hunting behaviour), and 'consummatory' (chunkier feed texture for eating behaviour) were studied as separate groups for comparison of their effects on fur-chewing. The fur-chewing of the mink was evaluated on a scale of '0- no damage; 1- tail sucking, hair damage on the tail tip, but no clear hair removal; 2- hair clipping/chewing over minor areas (<2cm diameter on body/neck or <1cm in tail); 3- hair clipping/chewing over larger areas or up to 1/3 of the tail; 4- widespread areas of damage or over 1/3 or tail without hair.' (MALMKVIST, J., PALME, R., SVENDSEN, P. et al, 2013) Access to biting ropes (hunting behaviour) reduced the incidence of fur-chewing on the tail from twenty-nine percent to sixteen percent. Chunkier feed (eating behaviour) reduced the incidence of fur-chewing on the tail in females only, halving it. Both of these alterations reduced the average fur-chewing score from 0.9 to 0.4 (independently from each other). These results suggest that both hunting and eating behaviour have an effect on the incidence of fur-chewing in mink (predatory species), and therefore this suggests that if we apply the same logic to chinchillas (herbivorous species), then increasing the time they spend foraging for (equivalent to hunting) and then consuming their feed, then the lower the incidence of fur-chewing should be. (MALMKVIST, J., PALME, R., SVENDSEN, P. et al, 2013)

Another paper regarding treating, or reducing, stereotypic behaviour, in fur animals (mink) discusses using selection (breeding). This paper does mention, however, that stereotypic behaviours often have a strong genetic factor in cause in some species, whilst being more dependent on the environmental stimuli in other species. (HANSEN, B., JEPPSEN, L., BERG, P., 2010) So the theories and results presented in such studies may not be directly transferable to another species, such as chinchilla. This was a very large scale study, involving 1484 mink dams, and the selection criterion for sample animals was based on the frequency of these stereotypical behaviours of the dam. (HANSEN, B., JEPPSEN, L., BERG, P., 2010) The study showed a percentage of stereotypical behaviours out of total observations, over the base population the result was 10+/-13.7, in the line selected for a high prevalence of stereotypical behaviours, the result was 13.9+/- 0.7, and in the line selected for low occurrence of stereotypical behaviours, the result was 4.3+/- 0.4. The study concludes that it is possible to reduce the frequency of these SB through the process of selective breeding. (SB being stereotypical Behaviour) however also recognises that there will always be a number of animals showing SB and total elimination by selection is very difficult and usually impossible. (HANSEN, B., JEPPSEN, L., BERG, P., 2010) And it is also posed that 'It is possible to change the animals' genetic potential for SB by selection, but it is the environment, including feeding and management, that determines to what extent genetic potential is utilized.' (HANSEN, B., JEPPSEN, L., BERG, P., 2010) Which suggests that to gain the most successful result of selectively breeding to reduce stereotypic behaviour incidence, we must also manage the environment of the animals appropriately, which implies that perhaps a mixture of both of the above behavioural and genetic methods of reducing stereotypical behaviour would have a greater impact.

A further research paper looks at the effectiveness of drug treatments (namely fluoxetine) on fur-chewing specifically in chinchillas. The study refers to the habit of fur-chewing as animals who repetitively chew their own fur down to approximately half the length of the hair shafts, most frequently on their flanks and sides. (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013) The authors also acknowledge a recent hypothesis that 'fur-chewing is a type of compulsive/impulsive behaviour and that stress plays a role in its development' and compares it to OCD (Obsessive compulsive disorder) in humans. This comparison is what led to the use of 'fluoxetine, a selective serotonin reuptake inhibitor (SSRI)' (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013) in this study, as it is a psychotropic medicine commonly used in OCD. Thirty- four chinchillas were studied, a relatively small sample size, all of which were affected by fur-chewing; these animals were separated into a control and treatment group. A 'dose of 10mg/kg/day of fluoxetine was selected for the chinchillas.'(GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013) And the drug was administered in corn syrup inside a raisin (used to disguise the bitter drug). The end results of the study showed that 'only ~46% of the fluoxetine treated animals showed a clear yet slight improvement in the fur condition.' (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013)The fur-chewing in the control groups showed 'increasing percentages of external signs up to 75 days of observations and a sudden decrease to nearly baseline levels at the end of the experiments', which couldn't be explained by the data of the study, however, the authors hypothesize that the 'disturbance of the keeper during the raisins administration and that a clear effect of fluoxetine was to prevent such an increase'. (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013) The study overall concludes that 'the use of fluoxetine in fur-chewing chinchillas showed limited success' and suggests exploring higher dosages in further studies. (GALEANO, M., RUIZ, R., FIOL DE CUNEO, M. et al, 2013)

2 g) Effects of Fur-chewing on the Fur-farming industry and its Economic Impact

As mentioned in the introduction section of this thesis, in 2015 the total export worth of fur in Europe was €573,412,403, meaning that problems causing economic impacts in this field (such as fur-chewing) can have enormous financial implications. (FUR INDUSTRY BY COUNTRY, 2017)

One study looked at the link between fur-chewing and reproductive ability of male and female chinchillas, which is a vital aspect of fur-farming, as in fur-production the animal itself is the product (as opposed to production animals like dairy cattle or laying hens, whose reproduction is considered a by-product). The study was focused on sexually mature domestic chinchillas (*Chinchilla lanigera*) who were proven breeders (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014) which were all categorised at the start of the study as either normal (non-fur-chewing) or '(1) slight – only a few tufts of hair are chewed; (2) moderate – one of the sides or hips is extensively chewed; (3) severe – both sides of the body or hips are chewed; and (4) very severe – all the fur in regions of the body that the animal can reach are chewed'(GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014) All of the animals, regardless of their condition, were kept as follows: 'The housing, environmental and management conditions were the same as those used in commercial breeding farms.' (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014) This suggests that the study should produce well-reliable results as the conditions were well standardized.

The results in table 5 represents the information gathered from semen samples from the chinchillas in the study, obtained via electro ejaculation. 3 samples were gathered from each male, and the averages of these values can be observed here.

Table 5 Selected characteristics (mean +/- SEM) of electro ejaculation procedure and semen of domestic *Chinchilla lanigera* showing different intensity of fur-chewing behaviour. (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014)

Characteristics	Fur-chewing behaviour				
	Normal (n=6)	Slight (n=3)	Moderate (n=3)	Severe (n=5)	Very Severe (n=7)
Semen Volume (μl)	42.9+/-5.9	53.3+/-15	68.3+/-31.8	40.0+/-12.7	36.1+/-10.2
Sperm concentration ($x10^6ml^{-1}$)	429+/-118	1116+/-296	1770+/-1628	1050+/-554	2193+/-686
Number of stimuli to achieve ejaculation	4.0+/-0.9	4.7+/-1.2	5.0+/-1.3	4.4+/-1.0	6.4+/-2.1
Electro ejaculation effectiveness (%)	96.7+/-3.3	91.7+/-8.3	75+/-14.4	88.3+/-7.3	84.4+/-7.4

There is no real demonstrable difference in any of the measured reproductive parameters of fur-chewing male chinchillas of any severity compared to the 'normal' chinchillas, especially when taking into account that no SD (standard deviation) is provided, and the SEM (standard error of mean) is quite large for the values of the data. (TABLE 5)

The results of table 6 below represent the information gathered regarding the reproductive success of fur-chewing female chinchillas compared to 'normal' chinchillas, however, unlike with the males results, they are not divided by degree of fur-chewing severity.

Table 6 Reproductive performance of normal and fur-chewing domestic *Chinchilla lanigera* females. (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014)

Parameter	Normal females	Fur-chewing females
Number of litters/female/year	1.4+/-0.05	2.2+/-0.5
Litter size at birth	1.9+/-0.08	2.2+/-0.2
Weaning success	88.9+/-4.1[a]	59.0+/-13.4[b]
Data are expressed as mean +/- SEM; normal females: 1452 females from six breeding facilities; fur-chewing females: n=15 animals. Weaning success is reported as the percentage of pups surviving through weaning (60 days of age); a vs b: ρ=0.05		

The number of litters produced by each female each year and their litter size was not affected by their fur-chewing behaviour. But it is also evident that the weaning success was lower in fur-chewers, even though, as the authors of the study note 'the weaning success of normal females was consistent with previous reports'. (TABLE 6) (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014) Obviously this has ramifications for welfare of the animals, as the fur-chewing seems to correlate with poorer mothering instincts/lower weaning success, and also has huge financial ramifications for the fur-farmers as the loss of so many pups means that a lower number of chinchillas will be raised to an appropriate age and size for slaughter for the pelts, leading to a decrease in profits, at the same time as losing the value of the fur-chewing mothers pelt due to the fur-chewing's direct damage. (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014)

A different study looks at the role that genetics plays in fur-chewing, and specifically looks at the cost of fur-chewing. The point is raised in this study that 'In the fur industry, chinchillas have a great commercial value due to the exceptional qualities of their fur, which reaches in average 2.5 cm of length, is silky, extremely soft, and firmly attached to the skin. In most mammals each hair follicle produces only one hair, but in this species each follicle produces between 24 and 80 hairs' (GONZALEZ, C., YANEZ, J., TADICH, T., 2018), which echoes the point I made in my introduction, about the fact that although chinchillas make up a relatively low percentage of the fur animals kept in Europe, they are still representative of a large part of the profit of the industry. 'The behaviour is more frequent in females, but can develop in both males and females usually starting at around 6–8 months of age. Fur-chewing does not only affect the animal's welfare, but also affects their skin, with the potential to impact large areas of their body. In consequence, affecting the fur quality, it also affects the fur price, since size and quality are the features that influence fur price the most' (GONZALEZ, C., YANEZ, J., TADICH, T., 2018) This is a vital point, especially when taken into account alongside the paper mentioned above which says that fur-chewing has a negative correlation with weaning success in female chinchillas. (GALEANO, M., CANTARELLI, V., RUIZ, R., et al, 2014) To analyse the link between fur-chewing and fur-price, data of 3007 animals was used, and to analyse the link between genetics and fur-chewing data of 9033 animals was used, so this was a very large scale study. (GONZALEZ, C., YANEZ, J., TADICH, T., 2018)

Table 7 Number of observations (N), mean, standard deviation (S.D.), variation coefficient (VC), minimum value (Min) and maximum value (Max) for each trait, considering fur-chewing (0 = not fur-chewer; 1 = fur-chewer) and sex (F = female; M = male) as categories. (GONZALEZ, C., YANEZ, J., TADICH, T., 2018)

	Fur-Chewing	Sex	N	Mean	S.D.	VC	Min	Max
Slaughtering weight *	0	F	1141	593.5	85.6	14.4	280	891
		M	993	585.6	80.9	13.8	313	870
	1	F	63	612.0	77.5	12.7	372	808
		M	67	592.3	81.2	13.7	406	817
Total/Mean			2264	590.5	83.2	14.1	280	891
Fur price **	0	F	1226	40.1	15.4	38.5	0	100
		M	1644	42.3	14.6	34.6	0	100
	1	F	57	23.2	11.9	51.2	0	54
		M	80	25.5	13.2	51.6	0	70
Total/Mean			3007	40.6	14.9	36.9	0	100

* Slaughtering weight is registered in grams; ** fur price is registered in US dollars.

According to the statistical analysis in the study, when adjusted to the ANOVA analysis, the mean price for a fur-chewing chinchillas pelt was US$21.1, whilst the mean price for a non-fur-chewing chinchilla was US$38, producing an enormous economic loss. (TABLE 7) Within this study it was also found that the incidence of fur-chewing was 5% in the population. (GONZALEZ, C., YANEZ, J., TADICH, T., 2018).

3. Materials and Methods of Research

3 a) Outline

Based on observations made from research literature regarding adrenal involvement in Fur-chewing Chinchilla, and the possibility of Cushing's-like disorders, I formed the hypothesis of an HPA (Hypothalamic-pituitary-adrenal) axis involvement in Fur-chewing. My theory was based on the Cushing's theory, with research results showing increased cortisol levels in fur-chewing chinchillas, and the negative feedback effect of cortisol on the hypothalamus and pituitary gland. (FIGURE 2) To test this theory, I decided to carry out experiments using gross dissection of chinchilla (including a general exam and focusing on areas of interest from research literature), followed by histopathological examination of target areas (slides taken from the hypothalamic-pituitary region and adrenal glands).

Via this research project I wished to compare the brain tissue (especially focusing on the HPA axis) of chinchillas which exhibit fur-chewing behaviour, with that of healthy members of the same species, based on information I researched for my literature review suggesting a link between increased adrenocortical function and fur-chewing in chinchilla, and the HPA axis working on a negative feedback mechanism, which would suggest an involvement of the full HPA axis is possible where adrenal disease affecting cortisol release is observed. (FIGURE 2)

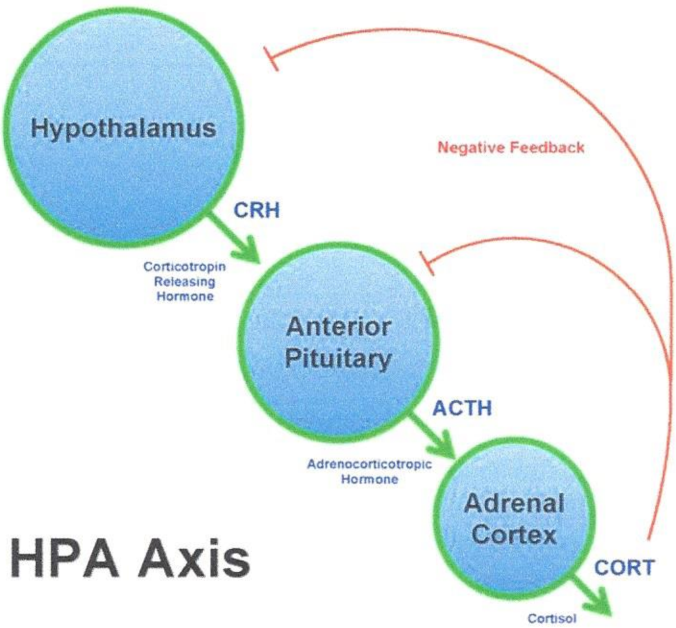

Figure 2: Basic hypothalamic-pituitary-adrenal axis summary (BrianMSweis, 2012) licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

I decided to carry out this specific research in this field whilst also exploring other potential aetiologies or findings mentioned in previous findings or discovered in our own PM (post-mortem) explorations. (Namely dental malocclusion and trichophagia)

In order to answer my question, I required data from both gross PM dissections (providing quantitative and qualitative data) and histopathological (qualitative) findings, of both healthy (control) and affected animals.

My aim with this study was to provide practically applicable information (and potentially provide evidence disproving some of the alternate proposed aetiologies surrounding this vastly under-studied field) in order to provide information for further studies into prevention and treatment of fur-chewing chinchilla, with an aim to improving the welfare of the affected animals.

This study is the most suitable approach to answering the questions posed in this research field, as the information simply cannot be gathered without using animals (replacement trials). And we utilised the 3R's (reduce, replace, refine) approach to research, minimizing the number of animals required, and minimizing the suffering of the animals used.

I designed the experiment based on information found in the research and writing of my literature review, highlighting areas of research which provide promising theories for aetiologies. Specimens for the research were euthanized by Dr Zoltan Orosi at Chinchilla farms in Hungary, and transported to the Department of Pathology at the University of Veterinary Medicine Budapest for the study. They were selected based on the farms culling requirements of fur-chewing (sick animals) and the control specimens were selected at random to prevent bias.

From our trial experiments, we refined our methodology and decided which areas of the data collection to focus on. We then carried out our preliminary experiments, which would have been followed by a collection of a wider sample range and data collection to provide accurate, comprehensive results. Unfortunately, this could not take place, due to the closure of university to students, and the lockdown of chinchilla farms due to the COVID-19 crisis. So in this section, I will discuss the trials, preliminary trials and 'suggested future trials'.

3 b) The Specimens

The chinchillas we selected for the trial are from farms where they are kept in 50x40cm grid floor area by 35cm high cages. These cages are stacked 4 storeys high. (FIGURE 3 AND 4) The water supply in the cages is constant with tap water and is supplied automatically from a central system.

Figure 4 : Chinchilla farm in Hungary picture 1

Figure 3 : Chinchilla farm in Hungary picture 2

The temperature in the barns is kept at 10-25 °C all year round (normally 12-14°C in winter and 20-22°C in summer) and the air humidity is kept below 70% (normally around 50-55%).

Artificial light is used in the barns to provide 10 hours of light every day. Within each room of the farm there are 100 females and 13 males. Each male can visit 8 females via a corridor in the back of a row of cages, however the females can't reach each other, and they are permanently separated.

These keeping conditions meet with the Hungarian legal regulations (32/1999. III.31), which in turn meet EU regulations.

With regards to nutrition, feed is provided ad libitum (as rodents, other than guinea pigs, will limit their consumption based on energy requirements); the feeders are filled up normally 2 times a week. The feed used is an 18mm diameter chinchilla pellet, which is custom made.

The babies are kept with the mothers for 42-50 days and after weaning and separation are kept in the cages of identical size individually until sale for pet-shops.

3 c) Data Collection

3 d) Dissection

3 d) i) Method

Trial experiment

At the start of each gross dissection we assigned the chinchilla with an ID number (beginning with 1 and ascending numerically), we then sexed the chinchilla, using ano-genital spacing (the most common method of sexing Rodents). (FIGURE 5 AND 6)

Figure 6 : Female Chinchilla sexing

Figure 5 : Male Chinchilla sexing

After this we carried out external measurements and observations: weighing, measuring body/head/ear/tail length. We also checked the oral cavity for malocclusions or other dental-disease associated lesions, and assessed the grade of fur-chewing in afflicted individuals: ' (1) slight (only a few tufts of hair are chewed); (2) moderate (one of the sides or hips is extensively chewed); (3) severe (both sides of the body or hips are chewed); and (4) very severe (all the fur in regions of the body the animal can reach are chewed)' (PONZIO, M., MONFORT, S., BUSSO, J., et al, 2012) We also carried out a full external examination, checking stage of development, state of nourishment, general condition, muco-cutaneous junctions, skin, feet, fur etc.

After the external examination we proceeded by laying the specimens in dorsal recumbency, cutting the attachment of the pelvic limbs to lay the specimens flat, and then skinning the specimens. Once skinned, we opened the abdominal and thoracic cavity, followed by an internal exam, looking for PM changes and gross pathological findings. Once any of these finding were recorded, we looked for specific findings, namely: adrenal hyperplasia and trichophagia (evidenced by hair in the GI (gastrointestinal) tract). Once these examinations were carried out, we removed the GI tract, and measured both the small intestine and large intestinal lengths.

We then took samples and fixed them in a 10% formalin solution. We took samples from various areas of the GI tract (stomach, small and large intestines), kidneys, adrenal glands, liver, skin, spleen etc.

Finally we removed the skull of the specimen, opened the dorsal aspect of the cranium, removed the brain and fixed this in the 10% formalin solution.

Throughout this PM inspection, the data was recorded in an individual data sheet (table 8).

Preliminary Experiment

After carrying out our trial experiments, we refined our methodology, and removed measurements which had too high a degree of human error to be of statistical value.

The methodology of the Preliminary round of experiments used the same methodology as the trial experiments, with the following exceptions:

We no longer measured the body/head/ear/tail length, as the measurements were influenced by too many variables based on how the body was positioned, where the examiner started measurements from, and the stage of post mortem changes (rigor mortis may affect these measurements).

The length of the GI tract was also omitted, due to similar reasoning.

The final change we made was during the removal of the skull of the specimen, we opened the dorsal aspect of the cranium, removed all extraneous tissues (skin, eyes, muscle etc.) and fixed the brain within the remains of the skull, for easier removal later, as it was found in the trial experiment that removal of the brain before fixation with formalin had destroyed some of the brains structure, rendering the histopathological slides useless.

3 e) Example Data Sheet for Chinchilla Dissections

Table 8 Example data sheet for Our Chinchilla dissections

ID (Number)		**Group**	
Sex (Male/Female)		**Age**	
Weight (g)			
Weight of brain (g)			
Fur-chewing (Yes/No)			
Grade of fur chewing (1-4) *			
Malocclusion observed (Yes/No)			
Adrenal Hyperplasia observed (Yes/No)			
Gross Pathology lesions observed (Organ, lesion, description)			
Samples Taken for Histopathology	lymph node (which: ______________) spleen liver stomach gut (small/large) pancreas kidney adrenal glands bladder prostate testis uterus ovary lung heart vessels thymus thyroid gland brain other (______) skin		
Histopathological findings			

*' (1) slight (only a few tufts of hair are chewed); (2) moderate (one of the sides or hips is extensively chewed); (3) severe (both sides of the body or hips are chewed); and (4) very severe (all the fur in regions of the body the animal can reach are chewed)' (PONZIO, M., MONFORT, S., BUSSO, J., et al, 2012)

3 f) Histopathology

3 f) i) Method

Trial Experiment

Using the organ samples taken in the above PM explorations, we produced and examined histopathological slides of the adrenal gland and the hypothalamic-pituitary region of the brain. To do this we used the following simple slide preparation technique.

- Tissue fixation in 10% buffered formalin solution
- Trimming and transfer to cassettes
- Dehydration in increasing concentrations of alcohol
- Clearing with solvent
- Embedding in paraffin, forming a block
- Sectioning using a microtome
- Sections scooped onto slides from warm water bath and dried, whilst excess paraffin melted.
- Staining with haematoxylin and eosin (haematoxylin 5 minutes, water 5 minutes, acid alcohol 5 minutes, wash with water, eosin 10 minutes, wash 5 minutes)

These slides were then examined (providing qualitative information) using a light microscope and images were captured using a microscope scanner. During this process we were looking for any major changes observable from the normal presentation described in the literature, especially regarding the brain (hypothalamus-pituitary region) and adrenal glands. Especially looking for signs of 'excessive adrenocortical hypertrophy.' (VANJONACK, W., JOHNSON, H., 1973) The reason for examining the HPA axis, rather than just the adrenal glands, was to research whether the reported 'significantly higher ($P< 0.001$) corticosterone plasma levels' (VANJONACK, W., JOHNSON, H., 1973) had any noticeable histopathological effect on the HPA axis, via the negative feedback effect.

Preliminary Experiment

The only difference made to the histopathological methodology from the trial experiments was to carry out fixation of the brain within the skull with extraneous soft tissues removed, rather than fixing the brain removed from the skull in the 10% buffered formalin solution. Meaning that during the trimming stage of slide preparation the fixed brain had to be removed from the skull. This was done to preserve the structure of the brain, as in the trial experiments, large areas of the brain had damaged structure due to the traumatic removal in the post-mortem examination, which rendered the results of the histopathological exam useless.

3 g) Analysis

During the gross pathological report the quantitative and qualitative data was gathered during the experiment using an individual data sheet for each specimen. (Table 8)

The quantitative data was then compiled and tabulated, as can be seen in the research results section of this study.

My qualitative data I categorized via content analysis, looking for trends and patterns in the observed data.

The histopathological slides were then examined, and any qualitative data was recorded alongside that specimens gross findings. Unfortunately, due to the COVID-19 crisis, I could not be present for histopathological examination, and therefore have included information provided by my co-supervisor in the research results section of the study.

4. Results

4 a) Gross Pathology

4 a) i) Trial experiments

Based on observations made from research literature regarding adrenal involvement in fur-chewing chinchilla, I formed the hypothesis of an HPA axis involvement in fur-chewing. To test this theory, I decided to carry out experiments using dissection of chinchilla (including a general exam and focusing on other areas of interest from research literature), followed by histopathological examination of target areas (samples taken from the hypothalamic-pituitary region and adrenal glands).

Initially we carried out some trial dissections, to allow us to refine our methodology, from this we decided to no longer collect data on body/head/ear/tail length, GI tract length. Also, as none of the trial experiment specimens had any hair in the GI (Gastrointestinal) tract (as a sign of trichophagia) we decided to not record Yes/No data for this field, rather, if this was detected in any of the specimens in the preliminary experiments, then it would be mentioned in the 'additional comments' section in the data sheet (TABLE 8)

4 a) ii) Preliminary experiments:

Table 9 : Table of results from Gross Pathology

ID	Sex	Weight	Fur Chewing	Grade	Dental Malocclusion	Adrenal Hyperplasia
1	M	489g	Yes	1	No (pale teeth)	No
2	F	553g	Yes	1	No	No
3	M	420g	No	N/A	No	No
4	F	497g	No	N/A	No	No
5	F	371g	No	N/A	No	No
6	M	419g	No	N/A	No	No
7	M	659g	Yes	4	No	No

8	F	699g	Yes	4	No	No
9	F	529g	Yes	4	No	No
10	F	578g	Yes	4	No	No
11	F	751g	Yes	4	No	No
12	F	569g	Yes	4	No	No
13	F	655g	Yes	4	No	No
14	F	639g	Yes	2/3	No	No
15	F	677g	Yes	2/3	No	No
16	F	587g	Yes	2/3	No	No
17	F	688g	Yes	2/3	No	No
18	F	709g	Yes	2/3	No	No
19	F	616g	Yes	1	No	No
20	F	506g	Yes	1	No	No
21	F	758g	Yes	1	No	No
22	F	639g	Yes	1	No	No

The data shown in the table above is that which was collected at gross pathological examination in the preliminary experiments.

Our Sample size included 4 control specimens (Non-fur chewing animals) and 18 fur-chewers. The 18 fur-chewers can be broken up into 3 groups, 6 mildly affected fur-chewers (grade 1), 5 moderately affected fur-chewers (grade 2/3) and 7 severely affected fur-chewers (grade 4). (TABLE 9) The fur-chewers grading was done based on the severity of presentation (categories as discussed on the data sheet) (TABLE 8) Grade 1 being the mildest, with only some chewed tufts. (FIGURE 7) Grade 2/3 being those with more extensive chewing on both sides of the body. (FIGURE 8) Grade 4 being the most severe, where all fur in regions of the body which can be reached chewed. (Figure 9)

Figure 7 : Grade 1 Fur-chewing Chinchilla

Figure 8 : Grade 2/3 fur-chewing Chinchilla

Figure 9 : Grade 4 fur-chewing Chinchilla

Ideally a larger control sample would be preferred; however, as we were using randomly selected specimens, from the culled animals, to prevent bias, and in order to meet the standards of the 3R principle, we didn't want to euthanize more animals unnecessarily.

The male to female ratio of the sample pool is also unequal, however this is due to the availability of specimens (the husbandry of chinchilla farms as described earlier requires only 1 male to serve 8 females) so our sample includes 4 males to 18 females (2:9 ratio), which for the chinchilla populations is a perfectly acceptable representation. (TABLE 9)

As can be seen from the table, none of the specimens in our data pool had signs of dental malocclusion or adrenal hyperplasia identifiable on gross pathology. (TABLE 9) The lack of any signs of dental malocclusion and in trial experiments the lack of any signs of trichophagia in the GIT (Gastrointestinal tract) (no hair evident in the GIT and no signs of Trichobezoars), contradict the studies from my literature review regarding dental issues and nutritional issues being the aetiological cause of fur-chewing. Dental issues have been linked to fibre deficiency and then to fur-chewing, for example 'Gastric trichobezoars: result of fur chewing and lack of dietary fibre' (GRANT, K., 2014). Clearly from our sample of 22 specimens, we found a 0% rate of dental malocclusion and signs of ingestion of hair, which contradicts the studies I discussed in the 'Nutrition and nutritional issues' section of my literature review.

From my literature review, several studies noted increased adrenal activity in fur-chewing specimens, such as 'adrenal hyperplasia was confirmed in all fur-chewing chinchillas' (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002) However, in my gross pathological exam adrenal hyperplasia was evident in 0% of specimens, and no visible difference between the adrenal glands of the control specimens and the fur-chewing specimens was detected, contradicting this study.

4 b) Histopathology

4 b) i) Trial experiments

The histopathological methodology from the trial experiments was changed for the preliminary experiments, to carry out fixation of the brain within the skull with extraneous soft tissues removed, rather than fixing the brain removed from the skull in the 10% buffered formalin solution. Meaning that during the trimming stage of slide preparation the fixed brain had to be removed from the skull. This was done to preserve the structure of the brain, as in the trial experiments, large areas of the brain had a damaged structure due to the traumatic removal in the post-mortem examination, which rendered the results of the histopathological exam useless.

4 b) ii) Preliminary experiments

After the collection of tissue samples in the gross pathological examination, the tissue was preserved and slides prepared in the method described in the methodology section of this study.

Unfortunately due to the timing of the COVID-19 outbreak, I could not be present for examination of the slides, and therefore cannot provide a comprehensive review, or scans of the slide sections. My co-supervisor, examined the slides of the hypothalamic-pituitary region and the adrenal glands and found no pathological abnormalities present to report on.

5. Conclusions:

Upon concluding my literature review, it was clear that there is a general lack of consensus on the aetiological cause of fur-chewing in chinchillas; several avenues of research have been explored, from hormonal disorder, to nutritional and dental issues through to stereotypical and behavioural issues. Unfortunately there isn't a wide range of studies and data in each of these research fields, meaning that there is no definitive answers regarding fur-chewing. The most researched field, with most data available, from the literature review was hormonal disorders, specifically related to adrenal function; therefore this was the area I decided to focus my research on.

On gross pathological examination of specimens in our sample, fur-coat changes typical of 'fur-chewing' were observed of varying severity. The absence of chewed fur in the gastrointestinal tract of 100% of fur-chewing chinchillas in our study contradicts some literature in the field (GRANT, K., 2014), whilst confirming the data from other studies (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002).

From gross pathology, 0% of our specimens had clinically observable dental malocclusion. This contradicts one of the 'traditional' theories for the cause of fur-chewing, which has been mentioned in studies suggesting that dental malocclusion causes a reduced dietary intake of crude protein and fibre, leading to fur-chewing as an alternate fibre source. (GRANT, K., 2014) However we must bear in mind that as we carried out no behavioural studies, we cannot guarantee that the fur-chewing was all carried out by the affected animal (rather an effect such as barbering in mice may occur, and we don't know the dental or nutritional status of cage-mates not included in the sample).

Our necropsy showed no signs of adrenal hyperplasia in 100% of our specimens, in direct contradiction of a previous study, which showed signs of adrenal hyperplasia in 100% of fur-chewing chinchillas studied (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002) Evidently this is qualitative data, and is quite unreliable, as histopathological examination is the only certain way to confirm if hyperplasia has occurred.

Unfortunately, due to the COVID-19 pandemic, the University was closed to students, and I could not complete my histopathological examination; however the reports from my co-supervisor found no evidence of hyperplasia or other pathological processes in the slides taken from the adrenal glands or the hypothalamic-pituitary region. This is contrary to the results of previous studies (TISLJAR, M., JANIC, D., GRABAREVIC, Z. et al, 2002) In ideal circumstances, we would have carried out further examinations to gain a larger, more representative sample range, and I would have been present to carry out histopathological examinations.

For a definitive and complete diagnosis a wider and more complete battery of clinical and laboratory tests would be required. On the basis of my studies we still cannot give an aetiological cause for fur-chewing. I would recommend further studies, and based upon the wide based research covered in my literature review, I would suggest focusing on adrenal function. I believe that an ideal experiment would involve clinical trials, measuring urine levels of glucocorticoids (minimally invasive measurements, with high levels of excretion) (PONZIO, M., MONFORT, S., BUSSO, J., 2004). Following the measurements of urinary glucocorticoids (in control and fur-chewing populations) the same process as followed within my experimental methodology would be followed. This way, the adrenal function of the living animal could be compared with pathological and histopathological data, providing a more complete data set, and providing a more reliable answer for the aetiological cause of fur-chewing.

6. Summary

The chinchilla (*Chinchilla lanigera*) is an exotic small mammal, of the rodent family, which is widely used in fur-farms, laboratory research, and increasingly is being kept as an exotic pet. Fur-chewing is a common disorder, affecting an average of 5% of chinchillas in one survey. Despite this causing huge financial losses in chinchilla farming (for the fur-industry and pet industry) due to decreased pelt value and weaning success, as well as being a welfare issue, there is no widely accepted aetiological cause currently. Several different aetiologies have been proposed and researched, including stereotypical behaviours, hormonal disorders and nutritional and dental disorders, although none of these studies have isolated a cause, and attempts to treat fur-chewing have been unsuccessful. From the literature review, we decided to focus on the HPA axis with a histopathological report due to several studies declaring results with adrenocortical hyperplasia and/or highly increased levels of corticosterone. During our gross pathological exam we found no signs of trichophagia in the gastrointestinal tract, and only very mild dental abnormalities in one animal, no signs of adrenal hyperplasia were noted in the control or fur-chewing animals. The histopathological examination also yielded no results indicative of adrenocortical hyperplasia or other pathologies of the HPA axis. From this study, I would rule out that a nutritional or dental aetiology is likely, with no evidence to support this, however we also cannot corroborate evidence of adrenocortical pathologies reported in other studies of fur-chewing chinchilla. Building from our results and those of reviewed literature I would recommend further testing, measuring urine corticosterone levels as this would provide results in living animals with the lowest possible stress level (in comparison to measuring plasma levels) therefore being more accurate, and concluding the study with pathological and histopathological examinations. This would provide a more conclusive, comprehensive result.

7. Appendices

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