BS – ritgerð Maí 2014

Effect of environmental enrichment on voluntary salt intake in horses

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Yfirlýsing höfundar

Hér með lýsi ég því yfir að ritgerð þessi er byggð á mínum eigin athugunum, er samin af mér og að hún hefur hvorki að hluta né í heild verið lögð fram áður til hærri prófgráðu.

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Abstract

The aim of this study was to evaluate whether horses consume a significantly different amount of salt from a salt block when a “toy” in the form of an empty plastic bottle was hung in their boxes compared to when no toy was presented to them. This would shed light on whether or not voluntary salt intake in horses housed under generally accepted conditions can be affected by a factor external to the horse and unrelated to salt requirement or balance. Twelve horses were observed for the total time of three weeks during which they had no access to a toy and then access to one according to a concurrent multiple baseline design. The data gathered was not normally distributed leading to a Wilcoxon test for matched pairs being carried out. Salt intake was significantly lower during the periods when the empty plastic bottle was available to the horses (T = 16, $P < 0.1$ and T = 15, $P < 0.1$, Wilcoxon’s test for matched pairs). Combined data from periods without toys and combined data from periods with toys showed an even greater difference with lower consumption when the bottles were available to the horses (T = 10, $P < 0.05$, Wilcoxon’s test for matched pairs). Throughout the study, no horse was found to eat an amount less than or equal to its estimated sodium requirements.

The results found in this study showed a clear tendency for horses to consume less salt when a toy was hanged in their boxes. The results also support assumptions made in previous studies stating that salt lick over-consumption in horses might be linked to lack of stimuli.

On a broader scale, these results call for a re-assessment of modern ways of keeping horses stabled rather than offer a solution to problems seen as a result of poor well-being in housed horses.

Acknowledgements

All my thanks go to my supervisor, Anna Jansson for her precious advice and help throughout the experiment itself as well as the writing of this thesis.

My sincere recognition goes to the staff members of Hólar University's department of aquaculture and fish biology for trusting me with their precision scale.

Þorvaldur Sævar Tryggvason gets my everlasting gratitude for proofreading and making suggestions as well as providing help with Microsoft Excel for Mac. I am also very grateful to Fabio Quaradeghini for his proofreading and assistance.

Last but not least, special thanks go to the rest of my family for their support and encouragement.

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1. Introduction

Domestication of animals is known to have effects on their natural behavioural patterns (Ratner & Boice, 1975). While housing horses is the most efficient way for humans to control them, this way of keeping these animals has often been shown to limit their social and kinetic behaviours as well as alter their natural feeding patterns. The way we keep the horse is closely intertwined with its welfare and it is therefore crucial for us to remember is that once we start managing it, we become responsible for the horse (Mc-Greevy, 2012). This responsibility includes the horse's nutrition and the effect it may have on its behaviour and welfare (Davidson, 2002).

The wild horse spends time browsing and grasing succulent forages composed in great part of water as well as of lipids, soluble proteins, structural carbohydrates and sugars. Contrasting to this, the domesticated horse is fed a range of feeds including cereals high in starch, forage high in moisture, long, fibrous stems composing hay, salt licks and water. Protein concentrates, dried forage and starchy cereals have been introduced to the horse's diet by man (Frape, 2010). Another example of modification in one of the horse's most fundamental behaviour patterns is the time spent feeding by feral or pastured horses compared to stabled horses. Feral or pastured horses appear to spend about 70% of their time foraging while housed horses only spend about 10% of the day eating (Marsden, 1995). Yet another difference is the fact that pastured or free-living horses seem not to choose to fast for over four hours at a time while some stabled horses may only have access to feed twice during the day with periods of fasting lasting for much longer periods, especially at night (Tyler, 1972; Doreau, Dubroeucq, Dumon St-Priest & Sauvage, 1978). Free range horses have shown a very varied diet both on a daily basis and on a seasonal basis as well as preferences for certain types of plants (Davidson & Harris, 2002). Unbalanced diets in horses have been linked to diseases such as equine metabolic syndrome (EMS), laminitis, equine degenerative myoencephalopathy and developmental orthopaedic disease (DOD) to cite only a few (Secombe & Lester, 2012).

1.1. Minerals

The horse's diet is composed in part of minerals (NRC, 2007). Minerals are generally classified into two groups; macrominerals, measured in g/kg or percentage of the diet and microminerals, measured in ppm or mg/kg of the diet. Although they constitute only a meager portion of the horse’s nutrition, minerals are crucial elements of the horse’s health and well-being. They participate in a multitude of bodily functions, counting physiological actions in acid-base balance, enzymatic co-factors, energy transfer and development of structural components. The greatest part of the minerals the managed horse requires are acquired through the ingestion of forages. Mineral concentration and availability in feeds depend on soil concentration, plant species and maturity, and harvest conditions. Since minerals will not be generated or broken down under ordinary conditions but must nevertheless be supplied in the ration, these variations need be considered when formulating horses’ diet as well as when determining an animal’s mineral status. As we can see, knowing and controlling the concentration of individual minerals in the diet is essential; but the ratio of all minerals should also be considered. The reason for this is as follows: minerals often influence other nutrients’ metabolism, absorption and/or excretion. In order to calculate mineral requirements for the horse, so-called balance studies that compare the amount consumed by the horse to the amount lost through faeces and urine (and occasionally sweat) are conducted. Failure to include sweat in this method can prove complex and inaccurate in some cases as horses, for example, lose sodium through sweat as well as through excrement. Another method has been to feed the animals uncommonly small or large quantities of the minerals in order to estimate at which point negative effects start to appear. The resulting numbers are then used to make dosage recommendations for the horse (NRC, 2007).

Electrolytes such as sodium, chloride and potassium are part of the macromineral group mentioned above and play a critical role in the horse’s metabolism. Together, they provide a controlled water based environment with particular acid-base balance and osmotic condition vital to cell function (Geor, Harris & Coenen 2013).

Salt as we generally refer to it is a combination of the two minerals and electrolytes sodium (Na) and chloride (Cl) (Alberts, Bray, Hopkin & Johnson 2010). It is necessary for horses to have access to salt and other minerals in different respective quantities in order for them to stay healthy and perform well (NRC, 2007).

1.1.1. Sodium

In the horse, correct functioning of the central nervous system, triggering of action potentials in excitable tissues as well as transportation of bodies across cell membranes all depend upon sodium (Johnson, 1995).

The skeleton comprises about 51% of the sodium contained in the horse’s body, the ingesta 12%, the skin 8%, the organs 2% and the blood and muscles together 11% (Meyer, 2001, as cited in NRC, 2007). Sodium is the major cation of extracellular fluid (ECF) (Dukes, 1993). Concentration of sodium in the extracellular fluid is on average 140mmol/L (Nelson & Cox, 2008). For growth, maintenance and late gestation, 1.6 to 1.8 g Na/kg dry matter has been suggested to be the ideal sodium concentration in the equine diet. For horses under moderate to heavy work, 3.6 g Na/kg dry matter is reported suitable (NRC, 2007). Sodium deficiency has been shown to lead to decreased skin turgor, slower rates of eating, decreased water intake, tendency to lick sweat contaminated objects and eventually eating abeyance (NRC, 2007). If sodium amounts are severely insufficient, muscle contraction and chewing becomes uncoordinated and an unsteady gait might be observed (Meyer et. al., 1984, as cited in NRC, 2007). It has also been observed that a period of five weeks on a diet low in sodium increases plasma aldosterone concentration as well as packed cell volume (Jansson, Johannisson & Kvart, 2010). When exercise intensity and ambient temperatures are increased, sodium loss through sweat increases which can lead to ion shortage (McCutcheon & Geor, 1998). Often, sodium concentration in natural feedstuff is seen to be lower than 0.1% which explains the effort to add salt (NaCl) to concentrates or to allow free-choice salt (NRC, 2007). Granted that enough water is available, sodium surplus will be excreted in the urine (NRC, 2007). The maximum tolerable concentration of sodium chloride in the diet has been set at 6% of total intake (NRC 2005, as cited in NRC 2007). High temperatures as well as lengthy exercise sessions are known to increase sodium necessity due to sodium losses through sweat. It is difficult to make precise sodium recommendations for exercising horses as different environments and activities have dissimilar effects on the horse's losses through sweat (NRC, 2007). In order to estimate sweat loss fairly precisely, one possibility is to measure weight loss after exercise. This number will help get an idea of how much sodium has been lost through sweat since it has been found that there are 2.8g of sodium in each 1L of sweat (Coenen 2005). Since the endogenous loss of sodium in the adult horse has been assessed to range between 15 and 20 mg/kg BW/day and if sodium is 90% absorbed, a good number for the maintenance requirement of the horse is 0.02 g Na/Kg BW. Under similar circumstances, the exercising horse thus needs (0.02 g Na x kg BW) + (3.1 g x kg BW loss during exercise) (NRC, 2007). Table 1. shows estimated amounts of NaCl lost through sweat according to different activities (Ekström, 2004).

Table 1. NaCl loss through various activities during different seasons (Ekström, 2004).

Activity	Conditions	NaCl loss g/100 kg BW
30 minute trot	Hot summer	8
High intensity exercise Standardbred/thoroughbred)	Winter/Fall	17
High intensity exercise Standardbred/thoroughbred)	Hot summer	27
Eventing (3 days)	Hot summer	35
Endurance (lowest - mainly trot)		45

1.1.2. Chlorine

As mentioned previously, chlorine is generally found following sodium in the diet of equids in the form of chloride. This extracellular anion plays an important role in acid-base balance as well as osmotic regulation. It is one of bile's fundamental composing elements. The production of hydrochloric acid, which is part of the gastric secretions crucial to digestion, depends partly on chloride. Consequently, if horses are being given salt, chlorine deficiency is not likely to occur. However, it should be known that in the case where horses are being fed sodium bicarbonate, metabolic alkalosis follows chlorine deficiency closely (Coenen 1988, 1991 as cited in NRC, 2007).

1.1.3. Water and sodium elimination

Excess water in horses is partly eliminated through renal excretion. Breakdown products of nitrogen (N) as well as sodium and potassium surplus are diluted in this water and evacuated out of the body forming urine (NRC, 2007). Horses fed concentrates have been shown to lose between 73-89% of their total water intake through renal excretion while horses fed on hay lost less than 60% of their total water intake through urine (Frape, 2010). The body's possibility to concentrate urea and highly soluble salts such as sodium and potassium is limited and more water will be needed in the diet if salt intake is high (Frape, 2010). Extrapolations from studies on other species indicate that an increase in the horse's dietary NaCl (salt) intake from 7.5 g/kg feed to 30 g/kg feed would increase dietary water intake to dry matter ratio from 2:1 to 3.5:1 (Frape, 2010). Output of urine is highly variable according to changes in biochemical and physiological stimuli. Protein, sodium or potassium renal solute load above the horse's requirements generally augment urine volume (Geor et. al., 2013).

1.2. Behaviour

In animals such as rats, salt appetite is believed to be linked to the hormone angiotensin although it is not yet known which part of the brain angiotensin affects in order for this appetite to be produced (Grossman, 1990). Ruminants and other herbivores seem to have an urge to ingest salt when in sodium deficiency. This appetite has been shown to be singularly accurate at controlling amounts ingested in order to balance this deficiency (Dukes, 1993). On the other hand, it should be noted that in some cases sodium appetite has not been observed to be the result of sodium deficiency or has sometimes been observed hours after the deficiency had been countered and sodium balance restored (Grossman, 1990). Nevertheless, this theory could be reinforced by the observation of feral horses visiting both natural and artificial mineral licks to ingest soil rich in minerals. Chemical analysis of the licks showed that the only mineral recurring in all of them in significant quantity was sodium. This leads one to believe that horses visited those licks specifically in an effort to satiate their sodium requirements (Salter & Pluth, 1980). Voluntary salt intake in athletic horses has been found to affect electrolyte regulation. Feeding frequency was speculated to be a potential factor affecting voluntary salt intake and therefore fluid and electrolyte regulation. A study led by Jansson and Dahlborn (1999) proved this speculation to be wrong as voluntary salt intake did not change significantly when horses were fed six times daily compared to twice daily. Another study showed that ponies fed a concentrate diet spend less time eating their rations but more time eating salt than if fed on hay (Willard, Willard & Baker, 1973).

Stereotypies are defined as repeated, non-changing behaviour patterns with no apparent aim or purpose. They have been linked temporally and physically to sub-optimal characteristics of the environment and have been used as an indicator of welfare (Mason, 1990). A few examples of equine stereotypies are weaving, crib-biting, wind-sucking, box-walking, self-biting, kicking stall and licking their environment (McGreevy, 2012). “Boredom” is often used as a general term to explain the occurrence of such behaviours (McGreevy, 2012). However, the term boredom should be used carefully as it has not been shown that horses show the capability of feeling bored in the way that humans do (Nicol, 1999). A more thorough assessment of the development of stereotypies has shown their causes to be variable between frustration, unavoidable stress or fear, restraint and lack of stimulation to give only a few examples (Mason, 1990). In equids, a link between the type of work asked of and performed by seventy-six French Saddlebred horses has shown to be linked to particular sterotypical behaviours (Hausberger, Gautier, Biquand, Lunel & Jégo 2009). It has also been established that diet clearly has a significant function in the development of abnormal oral behaviour both in horses and in other species (Nicol, 1999). When observing oral stereotypies, results indicate that prevention of natural grasing behaviour might be a major factor in the appearance of such behaviours with 50% less occurrence in horses allowed out to pasture for twelve hours daily (Christie et. al., 2006). On the other hand, weaving, a non-oral abnormal behaviour, seems to be the consequence of stable confinement and lack of available social interaction (Nicol, 1999).

Excessive salt intake can be one of many causes of psychogenic polydipsia (Buntain & Coffman, 1981). This may lead to wetter bedding due to more urine. The need to muck more often as well as add more bedding material to make up for humidity might then be required of stable managers. As can be seen in Table 2., bedding material in Iceland is relatively expensive (Lífland, phone call April 22nd 2014; KS Eyri, phone call, April 22nd 2014; Baldvin og Þorvaldur, phone call April 22nd 2014; Hestar og Menn, phone call April 22nd 2014). Economically, this means that if salt consumption in horses can be controlled and optimised for each horse, significant reductions in bedding material and workload requirements may be achieved.

Table 2. Prices of different bedding materials by Icelandic providers.

	Price isk.		
	Wood chips 20 kg	**Wood chips 26-28 kg**	**Woodchip pellets 16kg**
Lífland	2390	x	1590
KS Eyri	2390	x	1360
Baldvin og Þorvaldur	x	2590	1800
Hestar og Menn	1890	x	x

1.3. Previous Studies

One could expect that sodium appetite in horses relies on sodium requirements of individuals but the three studies that follow, although controversial on some points, all clearly show that, under the conditions studied, no such relationship between need and consumption can be found.

Schryver et. al. (1987) studied salt consumption in twelve sedentary thoroughbred, standardbred and quarterhorses of both sexes. All horses were fed at maintenance level. Voluntary salt consumption from salt blocks was measured weekly for up to forty-five weeks. The results showed ranges from 19g NaCl/day to 143g NaCl/day with a mean of 53g

NaCl/day. This number was considerably higher than both the calculated need of a 500 horse of kg 18.5g NaCl in the study and the 25g NaCl/day NRC (2007) recommends for a 500 kg horse.

In 1999, a study was led by Jansson and Dahlborn in order to find the effects of voluntary sodium intake and feeding frequency on fluid and electrolyte regulation in six standardbred geldings. The experiment lasted 50 days in total. A change-over design was applied where horses were fed six times per day during one twenty-five day period and twice a day during another twenty-five day period. The results were such that not only did feeding frequency have no effect on voluntary salt intake (and so consequently on fluid shifts and plasma aldosterone concentration) but voluntary sodium intake ranged between 0 and 62mg Na/day. This suggested that using a salt block as sodium source for horses might not be an optimal solution to avoid depletion.

A previous study (Guðbjartsdóttir, 2012) on salt consumption in Icelandic horses has shown that NRC (2007) recommendation numbers were generally far under the amounts eaten by the horses with an average of 33g Na/day. The experiment lasted for nine weeks during which salt licks were weighed weekly. Forty-seven horses were included in the study and divided into three groups according to their training frequency and intensity. No significant difference in voluntary salt intake was found between the groups but the results showed high individual variability. A significant difference in salt intake was found between weeks with salt intake decreasing during the first three weeks before increasing for the rest of the study's time. Variance was higher amongst mares than geldings but no significant difference was found between sexes. A small negative correlation was found between salt intake from salt lick and energy from feed as well as between salt intake from salt lick and dry matter ingestion and between salt intake from salt lick and sodium contents of the feed ration (Guðbjartsdóttir, 2012). The results observed in this study lead to think that the Icelandic horses kept at Hólar University have a general tendency to over-consume salt licks.

It has been shown that, in wood chewing horses, no exercise leads to an increase in voluntary salt intake. The authors hypothesised "boredom" to be the reason for this behaviour (Krzak, Gonyou & Lawrence, 1991).

Behaviour observations have shown that horses may play with plastic bottles hung in their boxes for up to 5% of their time (Bulens, Van Beirendonck, Van Thielen & Driessen, 2012). Interest in the items was higher when the horses had not been trained prior to the observations. The presence of hay or high quantity of bedding material decreased object-related behaviour and vice-versa. Biting of the object was observed more frequently when no hay was available to the horses. This is presumed to be related to frustration due to a lack of access to roughage or to an unmet need for fibres. Stallions or young horses were more interested in items hanged in their boxes than geldings, mares or older horses. In the same study it was shown that the interest of horses in the element introduced to them did not decrease from the first day to the last. However, we have to take into account that exposures to the toys did not last more than a week at a time (Bulens et. al., 2012).

To the knowledge of the author, no studies have been conducted on the effect of a toy or other environmental enrichment on salt or sodium consumption in horses.

2. Aim Of The Study

The aim of this study was to find out whether it would be possible to decrease salt intake in Icelandic horses housed at Hólar university by hanging a toy in the form of a plastic bottle in their boxes and therefore demonstrate that this behaviour may be linked to lack of stimuli.

2.1. Research Questions

Can an object such as an empty plastic bottle hung in the box of horses have a decreasing impact on their voluntary sodium intake and therefore reinforce the opinion that this behaviour is due to lack of other stimuli?

3. Material and Methods

3.1. The horses

Twelve Icelandic horses were used in this experiment. The group consisted of four stallions, two mares and six geldings of ages ranging from three to thirteen years old. Throughout the experiment period, their weights ranged from 318kg to 396kg with an average weight of 370kg. Each animal's weight was rounded to the closest whole number by the scale. The horses were picked randomly in a 24 horse stable according to whether or not a crib was at their disposal in the box and whether they had individual access to salt licks or had to share the lick with their neighbour.

The twelve horses were trained by seven different trainers each responsible for the training of one to three horses. The trainers were not chosen for the experiment but had been assigned to the horses during a certain period of time earlier in the year. Each horse was trained according to a programme suited to it and to the goals that had been set for it by its trainer or by the university's various training programmes. Four of the horses were trained by students aiming to complete a riding examination by the end of the experiment period. Six of the horses were being prepared for breed judging in the spring. Two horses were trained for leisure and competition and one was in light, basic training.

3.2. Organisation and conduct of the experiment

All horses were housed individually in one of Hólar University's indoor stables in the north of Iceland. The size and design of the horses' respective boxes varied slightly. Two of the boxes, one containing a stallion and one containing a gelding were 5.3 m^2. One box housing a gelding was 6.3 m^2 and all the others were 5.5 m^2. The stallions, five geldings and one mare were kept in boxes where physical contact with their neighbours was very restricted. Those boxes had horizontal metallic bars between which only the tip of the nose/mouth could get through. The rest of the group could pass their head and neck through two bars of metal and physical contact to their neighbours was therefore rendered relatively easy. All boxes allowed for visual contact of other horses. Bedding material consisted of woodchip pellets and was the same in every box. Each box was mucked every working day of the week and at least once during weekends. An average of 5.4 kg of bedding material was added to each box every two days except on weekends when no bedding material was added. The horses were fed three times a day; between 07.00 and 08.00 in the morning, between 11.00 and 13.00 and between 17.00 and 18.00 in the evening. All of them were fed hay and a vitamin and mineral supplement called “Racing mineral” produced by Trouw nutrition (Netherlands). All stallions, one mare and one gelding were fed different amounts of concentrate as a dietary supplement. Two types of concentrate were used, one called “Hnokki” produced by Fóðurblandan hf. (Iceland) and the other called Besterly Herbic Muesli and produced by Energys (Poland). One stallion was also given Isio 4 oil produced by Lesieur (France). All supplements were fed in the evening. Rations were calculated using the Norwegian computer programme “PC Horse” which takes race, gender, age, weight and training intensity and frequency into account. All horses had been fed according to the programme since the 15th of January 2014 with re-evaluation of respective rations every two weeks. PC Horse was used in order to achieve or maintain body condition score three on the 0-5 scale (Guðrún Stefánsdóttir, & Sigríður Björnsdóttir, No Year). Table 3. and 4. show individual rations as well as total sodium amount of each horse’s diet throughout the experiment time. Every box had an automatic water supply. White salt licks of 10kg were provided to each horse with ad libitum access. Previous to the experiment, all horses had had ad libitum access to salt for at least one week to ensure that no horse would be in deficiency when the measurements would start. The salt licks were fixated on stands through a hole in the middle of the block. For three horses no stand was available so a small chain or rope was used to attach the block to the box. Each horse included in the experiment was let out in a paddock with other horses – alone in the case of the stallions – for about an hour every day.

A concurrent multiple baseline design was applied to the experiment. During the first week, the horses had no access to environmental enrichment of any kind. During the second week, the horses had access to environmental enrichment in the form of an empty plastic bottle attached to the bars of their respective stalls with hay bale strings. The third week of the experiment was divided into two periods of three and a half days where the horses first had no access to the bottles and then had access to them again. Each period was respectively nominated A (no bottle), B (bottle), C (no bottle) and D (bottle).

After each period of time, salt licks were weighed using a precision scale (model Marel M1100, Iceland) and returned in place immediately. This was done between 17.00 and 19.00 each time except after the first three and a half day period when it was done at noon.

Each horse was weighed weekly using a Gallagher smartscale 300 (New-Zealand) provided by the University of Hólar.

During period B, behaviour samplings were conducted continuously for one hour every two days in order to see if the horses were interested in the plastic bottles at all. The samplings were conducted at different hours each time.

Table 3. Individual rations of feed and amounts of Na in feed for periods A (no bottle) and B (bottle).

12.03.2014 - 26.03.2014 Feed information						
Horse	**Hay kg**	**Racing Min. g**	**Best. Herbic g**	**Hnokki g**	**Oil**	**Na in feed g/day**
1	6	130	0	0	0	11,82
2	6	120	250	0	0	12,64
3	7	120	0	0	0	12,84
4	9	80	250	1000	0	19,40
5	5,5	120	0	0	0	10,86
6	8	80	1000	500	0	19,45
7	5,5	120	0	0	0	10,86
8	7	120	0	0	0	12,84
9	5	130	0	0	0	10,50
10	7,5	120	250	750	0	17,62
11	9	80	500	1000	150	20,52
12	8,5	80	250	0	0	16,86
Average	7,0	108	208	271	13	14,68

Table 4. Individual rations of feed for periods C (no bottle) and D (bottle).

26.03.2014 - 02.04.2014 Feed information						
Horse	**Hay kg**	**Racing Min. g**	**Muesli g**	**Hnokki g**	**Oil**	**Na in feed g/day**
1	6	130	0	0	0	11,82
2	6	120	250	0	0	12,64
3	7	120	0	0	0	12,84
4	9	80	250	1000	0	19,40
5	5,5	120	0	0	0	10,86
6	8	80	1000	500	0	19,45
7	5,5	120	0	0	0	10,86
8	7	120	0	0	0	14,15
9	5	130	0	0	0	10,50
10	7,5	120	250	750	0	17,62
11	9	80	500	1000	150	20,52
12	8,5	80	500	250	0	16,86
Average	7,0	108	229	292	13	14,79

3.3. Composition of hay, concentrates and salt lick

The hay fed to the horses during the experiment was harvested on the 27th of July 2013. It was then packed into square bales on the 30th of July 2013. Chemical analysis of the hay was performed by the Agricultural University of Iceland (Landbúnaðarháskóli Íslands). Table 5. shows information gathered after chemical analysis.

Table 5. Chemical analysis of the hay.

Hay				
Digestability %	**FEm in kg**	**Dry matter %**	**Fibre NDF g kg**	**Na g/kg**
63	0,69	63,3	552	1,3

Results for phosphorus (P), magnesium (Mg), potassium (K), selenium (S), iron (Fe), mangan (Mn), zink (Zn) and copper (Cu) were also provided by the chemical analysis of the hay. These numbers will not be included in this essay.

The amount of sodium in the different concentrates fed during the experiment was of 4 g/kg, 30 g/kg and 4.5 g/kg for hnokki, racing mineral and besterly herbic respectively.

The sodium and chloride composition of one kilogram of white salt lick was 99% dry matter, 390g Na (sodium) and 600g Cl (chloride). Other elements such as selenium (S), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), iodine (J) and cobalt (Co) are also components of the licks but their amounts will not be included in this study.

No sodium is found in Lesieur's Isio 4 oil (Lesieur, n.d.).

Each horse's respective sodium maintenance requirement was calculated for each period of the study and is shown in Table 6.

Table 6. Individual calculated sodium maintenance requirements for each horse throughout the experiment (based on NRC, 2007).

Na maintenance requirements g/day			
A (no bottle)	**B (bottle)**	**C (no bottle)**	**D (bottle)**
7,160	7,100	7,120	7,120
7,400	7,460	7,520	7,560
6,440	6,480	6,400	6,360
7,540	7,660	7,500	7,600
7,480	7,440	7,500	7,500
7,640	7,740	7,720	7,760
7,520	7,460	7,460	7,480
7,320	7,200	7,420	7,380
7,500	7,480	7,400	7,440
7,220	7,300	7,240	7,300
7,460	7,580	7,720	7,740
7,680	7,860	7,800	7,920

3.4. Statistical processing

All calculations were performed on the computer programme Microsoft Excel.

Since the data was not normally distributed, a simple t-test could not be conducted. It was therefore decided to use the Wilcoxon test for matched pairs. A series of three tests were conducted; the first one to find if there was a difference in median between period A (no bottle) and period B (bottle), the second one to find if there was a difference in median between period C (no bottle) and period D (bottle) and the third one, combining period A (no bottle) and C (no bottle) into one period and period B (bottle) and D (bottle) into another and analysing those two periods.

Spearman rank correlation coefficients were calculated between the amount of sodium contained in the feed and the amount of sodium voluntarily ingested and between the amount of hay fed to the horses and voluntary salt intake.

4. Results

4.1. Behaviour sampling scans

Table 7. shows that apart from two, all horses were seen interacting with the bottle in some way during the behaviour sampling scans. Different behaviours such as nipping, biting and simply moving through touch of the nose/mouth were seen.

Table 7. Records of interaction or non-interaction of horses with bottle during behaviour sampling scans.

Horse	Interaction observed
1	Yes
2	Yes
3	Yes
4	Yes
5	Yes
6	Yes
7	Yes
8	Yes
9	No
10	No
11	Yes
12	Yes

4.2. Descriptive statistics

Table 8. shows the consumption numbers for NaCl throughout all periods of the experiment.

Table 8. Salt lick consumption of the horses throughout all periods.

	Salt lick consumption				
	Period A no bottle	Period B bottle	Period C no bottle	Period D bottle	All
Average NaCl consumption g/day	133	97	109	78	104,25
Median	106	83	95	69	85
Minimum NaCl consumption g/day	69	31	36	34	31
Maximum consumption g/day	223	202	214	176	223
Range g	154	171	178	142	192

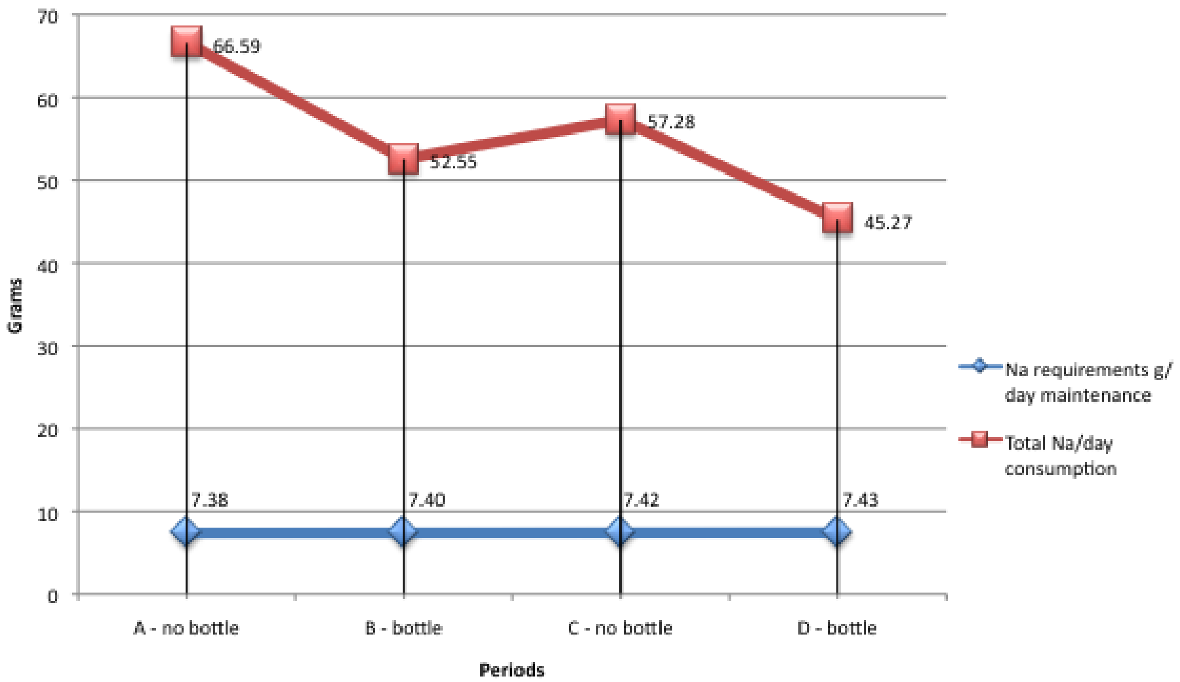

Figure 1. Evolution of sodium intake and calculated maintenance requirements during each period.

Figure 1. shows the difference between average daily sodium requirements of the horses and average daily sodium intake of the horses comprising sodium from salt lick and from feed.

A decrease in sodium intake during period B (no bottle) was followed by an increase shown during the time through which the bottle was removed - Period C (no bottle) - followed by a decrease once the bottle was placed back in the box - Period D (bottle). Figure 2. shows the individual consumption of the horses throughout the experiment.

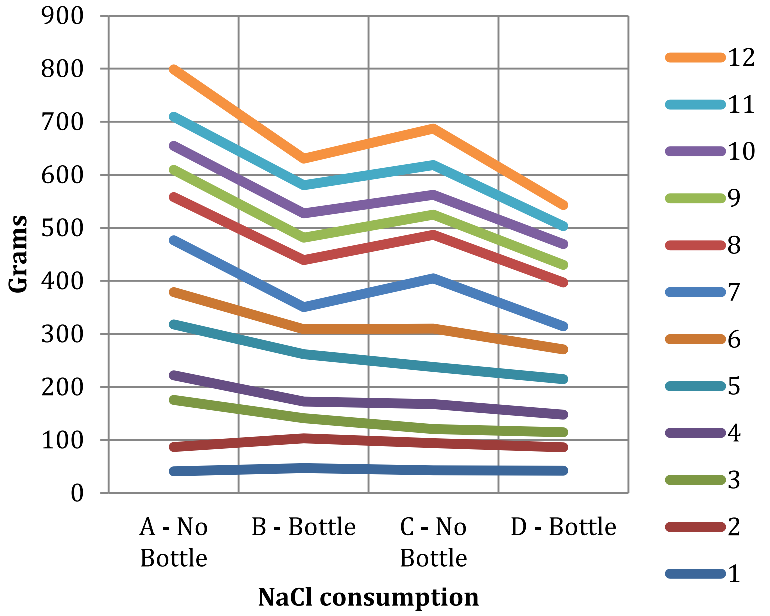

Figure 2. Stacked graph of individual NaCl consumption of the twelve horses during each period

This graph shows the tendency of the consumption to decrease among with high individual variability. It should be noted that the values of the y-axis are the sum of the consumption of all previous horses. Each line simply represents an individual's consumption pattern.

According to the results seen in table. 9., at no time did a horse consume less than four times its daily maintenance sodium requirements. At the maximum, one horse's sodium intake was observed as high as 13,7 times its daily requirements.

Table 9. Results shown by dividing actual sodium consumption by maintenance requirements calculated in table 6.

Number of times NRC maintenance requirements for Na fit in actual consumption of Na				
	Period A no bottle	**Period B bottle**	**Period C no bottle**	**Period D bottle**
Minimum	5,78	4,14	4,19	4,37
Maximum	13,73	12,14	12,64	11,20
Range	7,95	8,00	8,45	6,83
Average	9,06	7,10	7,66	6,08

The Wilcoxon for periods A (no bottle) and B (bottle) had the null hypothesis H_0 state that there was no difference in median salt intake between period A and period B. The hypothesis H_1 stated that there was a difference in median salt intake between period A (no bottle) and period B (bottle). The Wilcoxon test gave us the result $T = 16$. The value for N was 12 (there were twelve horses in each period). After consultation of the table of the probability distribution of *T*, it was found that the critical value at level of significance $P = 0.1$ is 17. Since $P < 0.1$. The null hypothesis H_0 was rejected and it was accepted that there was a difference in medians between periods A (no bottle) and B (bottle).

For periods C (no bottle) and D (bottle), the null hypothesis was that there was no difference in median salt intake between periods C (no bottle) and D (bottle). The hypothesis H_1 was that there was a difference between median salt intake between periods C (no bottle) and D (bottle). The Wilcoxon test gave us the result $T = 15$. The value N is still 12 and therefore the critical value at level of significance $P < 0.1$ is 17. The null hypothesis was rejected and it was accepted that there was a significant difference between the two periods.

A third Wilcoxon test was calculated after having combined periods A (no bottle) and C (not bottle) into one period and periods B (bottle) and D (bottle) into another in order to have a broader image of difference in salt intake during the times when horses had no access to a toy and the time when horses did have access to a toy. This time the Wilcoxon test gave us $T = 10$ which led to the critical value of 13 at $P < 0.05$. Again, the null hypothesis H_0 was rejected and it was accepted that there was a difference in medians between the time when the bottles were hung in the horses' boxes and the time when they were not.

Spearman Rank Correlation Coefficients found no correlation between the amount of sodium contained by the feed given (hay, concentrates and racing mineral) and the amount of sodium ingested through the salt lick or between the amount of hay fed and the amount of sodium consumed through salt lick.

5. Discussions

As can be seen in Table 9., average salt intake from salt licks per day was lower during the periods in which the plastic bottles were hung in the box with decreases of 36 g and 31 g during periods B (bottle) and D (bottle) respectively. Medians also showed a clear decrease during periods with bottles. Minimum salt consumption per day seemed to decrease when the toy was hung although the difference between period C and D was not considerably large (2 g NaCl/day). On the other hand, maximums were clearly higher when no toy was provided to the horses and especially so during the second part of the experiment.

Figure 1. shows us these numbers graphically and emphasizes the increase in voluntary salt intake when the plastic bottle was removed from the horses after period B (bottle). An increase in voluntary salt intake when the plastic bottle was removed during period C (no bottle), although not reaching as high as the numbers observed during period A (no bottle), was clearly visible (see figure 1. and table 9.). Conjectures of reasons for which it did not reach the same level as the previous bottle-less period could be that the bottles had a lasting effect on the horse's intake or that, as in the first three weeks of Guðbjartsdóttir's study (2012), the horses were in a period of slow consumption reduction.

While a general decrease in salt consumption was observed in the presence of the bottle, the results showed a minimum staying quite high with no time during which any horse saw its consumption go under four times its calculated maintenance requirements (see table 8.). It is difficult to draw conclusions from this since losses for example through sweat due to training were not calculated here.

Four horses saw their intake increase between period A (no bottle) and period B (bottle) and three between periods C (no bottle) and D (bottle) (see figure 2.). An interesting aspect of these increases was that in all cases but one, the horses seeing their intake increase were the smallest consumers during the previous bottle-less period. The only horse which was not a small consumer but still saw its intake rise was the horse being the least trained (in light basic training). This may reinforce the results presented by Krak (1991) where unexercised horses saw their voluntary salt intake increase. Here the bottle would not be enough to counter the frustration or boredom felt by the horse when its kinetic needs are limited. This particular horse was observed actively interacting with the bottle and was the youngest gelding in the group. This is somewhat converse to the results recorded by Bulens et. al. (2012) where stallions and young horses seemed more interested in the items hung in their boxes than geldings, mares and older horses.

The significant levels between respective periods were $P < 0,1$ which, as mentioned earlier, means that there was over 90% chance that our results are not due to coincidence. While this was enough to reject the null hypotheses stating that the medians were not significantly different, it would be highly valuable to see the same study conducted on a bigger scale. This would perhaps tone down individual variability effects or, on the contrary, show how important they are when it comes to voluntary salt intake. In this study, once the periods without bottles and with bottles were combined into two periods instead of four, the significant level showed $P < 0,05$ which shows much higher and clearer significance. This supports the need for a similar experiment conducted on more horses and for a longer period of time.

Throughout the study, a much higher average consumption of both salt (104g NaCl/day) and sodium consumption (41 g Na/day) were observed compared to both the numbers found by Schryver et. al. (1987) (53g NaCl/day) and in the previous experiment conducted on Icelandic horses by Guðbjartsdóttir (2012) (33g Na/day), Contrary to results found by Jansson and Dahlborn (1999), the sodium requirements of each of the horses in this experiment were not only met but exceeded and only three horses did not see their sodium requirements met by feed only. All three were receiving between 5 and 5.5kg of hay per day (the lowest rations in this study). Throughout the study, the horses were seen ingesting between 4.1 and 13.7 times the amounts they required for maintenance. These results are in line with the ones found by both Schryver et. al. (1987) and Guðbjartsdóttir (2012) although on a more inflated level.

The difference between the present results and the results found by both Jansson and Dahlborn (1999) and Schryver et. al. (1987) may be due to factors such as race, environment, time of year, feeding level and/or climate. The difference between the numbers found by Guðbjartsdóttir (2012) and the present ones may be due to the difference in number of horses included in the experiment, housing or the fact that in Guðbjartsdóttir’s (2012) study, the horses had access to 2kg salt licks but not 10kg salt licks as they had in the present experiment. Different training programmes might also have been a factor. It should also be noted that the horses observed by Jansson and Dahlborn (1999) were athletic horses under conditions of heavy training while the horses in the experiment led by Schryver et. al. (1987) were unexercised. The intensity of exercise also influences daily feed allowances in managed horses which may lead to more time spent feeding by horses being trained at high intensity as the ones seen in Jansson and Dahlborn's experiment. The time spent on feed intake behaviour might therefore have an effect on daily salt intake.

It would without doubt be interesting to see studies similar to this one performed on a greater number of horses and offering a longer time-span both in Iceland and in other countries in order to confirm or disprove the results recorded here. A similar study where behaviour would be recorded at all times would indubitably contribute invaluable information about horses' behaviour when stabled as well as perhaps provide a clearer image of the motivation of suboptimal salt intake behaviour. Since such high variability has been seen between horses and studies, and if similar results to the ones found in the present one are observed in the future, it would interesting to see different enrichments lead to different results. Perhaps for horses or conditions such as the ones encountered in Jansson and Dahlborn's study (1999), offering salt in different forms (loose, liquid) might be a better solution. Enrichments that stimulate voluntary salt intake from salt licks could also be valuable.

A thorough study including the factors seen in the present one but also keeping records on water intake and urine loss might prove very valuable for the common horse owner. Indeed if, as stated by Geor et. al (2013), sodium renal solute load above the horse's requirements lead to higher urine volume, it might be that a lot of bedding material could be spared as well as work mucking stables. If the introduction of enrichment such as a simple empty plastic bottle was found to affect urine output significantly through salt intake, an economical aspect would be added to the welfare aspect of the present results.

Another potentially interesting study would be the assessment of sodium intake of horses under natural circumstances. While they are known to visit both natural and artificial salt licks and that amounts consumed by these horse are obviously enough for depletion not to be observed, it is not known whether this consumption is anywhere close to individual optimal consumption (Salter & Pluth, 1980). Although, the simple fact that those horses do visit the licks is proof that they have some kind of sodium appetite preventing them from dying of depletion Jansson and Dahlborn's (1999) study shows that this is not always the case in domesticated horses. Also, this study as well as the ones led by Shryver (1987) and Guðbjartsdóttir (2012) clearly show a tendency in domesticated and housed horses to over-consume salt. If natural conditions indeed see the horse consume amounts close to its optimal needs, it might be argued that the reason for which the various studies referred to in this paper (Schryver et al., 1987; Jansson & Dahlborn, 1999; Guðbjartsdóttir, 2012) as well as the present one might not show such scattered results is that domestication conditions of horses kept in stables and/or trained for various reasons have altering effects on the horse's normal and natural regulation of sodium intake.

On a broader scale, the results mentioned above as well as research on stereotypies can be used to question the way horses are generally kept by man.
As Krzak et. al. (1991) had assumed, over-consumption of salt licks seems to be an indicator of lack of stimuli resulting into redirected, degraded behaviour due to limitations inflicted upon the horse by the way they are kept. This, along with the development of stereotypies showed in horses due to confinement and lack of social interaction (Nicol, 1999), prevention of natural grasing behaviour , frustration due to an unmet need for fibres (Bulens et. al., 2012) and stressors such as high intensity training should lead us to try and rethink the way we keep and manage our horses (Christie et. al., 2006; . Here, the over-consumption of salt licks and the fact that the presence of an environmental enrichment in the form of a bottle seems to decrease this over-consumption is yet another indicator of the need to re-assess the way we keep and manage horses. Environmental enrichment such as a bottle should therefore not be seen as a solution to problems such as over-consumption of salt but only as a very small compromise between the way we keep our horses and the way they should be kept in order for their needs to be met optimally.

6. Conclusion

The results revealed by this study confirm that voluntary salt intake by horses can to some extent be affected by a factor external to the horse and unrelated to salt requirement or balance. They also clearly show that the use of environmental enrichment in the form of an empty plastic bottle hung in the box of horses has the effect of decreasing their voluntary salt (sodium) intake. Although this environmental enrichment could be seen as a compromise between ideal management of horses and generally accepted methods of keeping horses stabled, this should not be seen as a solution but should raise our awareness to the fact that modern horsekeeping methods are not optimal to the well-being of the horse.

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8. Table of Figures

9. List of Tables