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Changes in eating time, chewing activity and dust concentration in horses fed either alfalfa cubes or long-stem hay

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Abstract

Background: Chewing is an essential physiological process in horses; yet, the physical form of feeds may affect their chewing and feeding behaviour with consequences for equine digestion and health.

Objectives and methods: The present study evaluated the potential of a commercial forage cubes made from alfalfa and mixed meadow grasses to maintain chewing activity when compared with a traditional long and fibre-rich hay. An additional aim was to measure the dust formation during feeding. The experiment was a crossover design with six horses (11 ± 4 years old, mean ± SD), fed with 5 kg (as-fed basis) of their feed (long hay or alfalfa cubes) overnight. Eating and chewing activity was measured using the EquiWatch system with a sensor-based halter.

Results: Data showed that by feeding the same amount of feed overnight, cube feeding led to 24% shorter eating time (on average 67 min less) and 26% fewer total chews compared to the long hay. On an hourly basis, horses also spent more time eating and chewing the long hay compared to the cubes. The cube feeding led to a higher dust concentration of the inhalable fraction (<100 μm), but not of the thoracic (<10 μm) fraction. Nevertheless, average dust concentrations were generally low for both cubes and hay, whereby both were of sound hygienic status.

Conclusion and recommendation: Our data suggest that feeding alfalfa-based cubes overnight generated shorter eating time and less chews than the long hay without major differences in the thoracic dust. Therefore, because of the decreased eating time and chews, alfalfa-based cubes should not be fed a sole forage source, especially when it is to be fed without restriction.

KEYWORDS
alfalfa, chewing activity, dust concentration, green fodder cube, horse, roughage alternative

Viktoria Petz and Ratchaneewan Khiaosa-ard contributed equally to this study.

1 | INTRODUCTION

The feeding of horses strongly depends on forages. General recommendations are that horse diets typically should contain not less than 1.5 kg long-stem hay per 100 kg body weight (GfE, 2014), preferably first cut and harvested at late maturation stage, thus with a high fibre content. However, due to a scarcity of such hay, other forages, mainly alfalfa and early-maturity grass hay processed in the form of cubes and pellets, have become alternatives in the equine feed market. Forage cubes have advantages over long-stem forages in terms of reducing space for storage and transportation, ease of handing, reducing wastage by the horse and promoting intake and lowering hay belly appearance (Lewis, 1996). Moreover, compared with pellets, the cubes have several advantages for horses. Although pellets largely lack the physical properties, with cubes the forage is coarsely chopped and then mechanically compressed into 1.25–2-in. cubes. It has been suggested that forage cubes can be a nutritive substitute for long-stem hay (Coleman, 1999), which is supported by collective data on digestibility and nutrient utilization (Potts et al., 2010; Younglove et al., 1994). However, it is not clear whether forage cubes are suitable as a complete substitute for hay to maintain the satisfactory chewing behaviour of a horse.

Indeed, chewing activity is important for particle comminution, sufficient salivation, taste perception and dental and digestive health as well as for the satisfaction of the horse's feeding behaviour and welfare. The physical form of feed affects chewing and feeding behaviour. Long-stem forages increase the time spent on eating and chewing (Ellis, 2010; Gordon et al., 2019) and promote mandibular motion and thus the dental health and proper digestion in comparison to pellet feeds (Bonin et al., 2007). Although some works have been performed on pelleted feed as shown in a review (Ellis, 2010), the empirical data on chewing behaviour due to feeding pelleted and cubed forages are generally lacking in horses. Although one might expect values similar to those for pelleted feed for cubed feed, the forage is ground prior to pelleting. The particle size of cubed forage is much larger than pellets and, therefore, may be better at stimulating chewing. Still, a common concern is that horses will eat cubes faster than loose and long-stem hay (Lewis, 1996; Coleman, 1999). Besides increasing choking risk (Lewis, 1996), a rapid rate of consumption of cubes may decrease the chewing activity of horses. Determination of eating and chewing behaviour is important to conclude whether cubed forage can be a substitute for long-stem hay.

Hay can be a major feed source for dust particles and fungal spores that affect respiratory health in horses. One main advantage of cubed forages, if made properly, is minimal dust during feeding (Lewis, 1996; Coleman, 1999). For this reason, cubed forage is recommended for horses with respiratory diseases. To our knowledge, the advantage in reducing the airborne particulates has not been substantially investigated either. Furthermore, variations in the choice of forage, cube size, and the making of forage cubes could affect the physical properties and, therefore, the dustiness when eaten. Accordingly, in the present study, we compared a commercial cube made of alfalfa and mixed meadow grasses with a common long-stem hay in terms of eating and chewing and dust concentrations related to feeding. We hypothesized that feeding the alfalfa and mixed meadow grasses cube would reduce eating time and chewing activity, whereas it would benefit in terms of reducing dustiness related to feeding.

2 | MATERIALS AND METHODS

2.1 | Test horses

The project was carried out at the Aspen Ranch in Salem, Baden-Württemberg, Germany. Six privately owned horses were enrolled in a crossover study with two test feeds in two experimental periods (Table 1). There was a 2-week washout period between the two experimental periods. The age of the test horses ranged from 4 to 16 years, and their height ranged between 140 and 160 cm. There were four Quarter Horses, one Paint Horse and one Irish Tinker. Their weight ranged between 440 and 544 kg as estimated with the following formula with an error of about 5% (Carrol & Huntington, 1988; Frape, 2010): Body mass (kg) = chest circumference (cm)^2 × body length (cm)/11,877.

The horses were kept together in groups in the pasture during the day and spent the night in individual stalls when they received 5 kg (as fed) of their test feed, which was equivalent to 4.5 ± 0.2 kg dry matter (DM) of hay and 4.3 ± 0.2 kg DM of cubes.

2.2 | Test feeds, nutrient analyses and feeding

The experimental hay was a stem-rich long hay, supplied from a farm in the Lake Constance region (Baden-Württemberg, Germany). The hay was a first-cut meadow hay, consisting mainly of grasses, such as meadow fescue (*Festuca pratensis*), timothy, (*Phleum pratense*), tall oat grass (*Arrhenatherum elatius*) and cocksfoot (*Dactylis glomerata*), and harvested in a late-maturity stage and sun-dried. A sensory analysis of the hay fed during the experiment indicated that hay was in a sound hygienic status (dry, light hay odour free of unpleasant smells, green–yellowish colour without visible impurities, such as weeds, moulds, sand or dust). Forage cubes (3 cm × 3 cm with long fibre) made of alfalfa and mixed meadow grasses (1:1) were a commercial product (M^2 Wafer Basic, Food4Horses, Romagna, Italy). Horses were offered either 5 kg hay or cube (as fed basis) between 1930 and 2030 h. Test feeds were analysed for their chemical composition according to the VDLUFA method (Naumann & Bassler, 2012), and the chemical composition is shown in Table 2. Horses received 300 g of oat grain at approximately 0715 h followed by free access to hay and grass throughout the day. The horses were acclimated to both types of feed 14 days prior to measurement. Both test feeds were well received by the horses who consumed all of their offered feed during the course of measurement.

2.3 | Measurement of eating and chewing activity

These measurements were carried out overnight right after test feed provision time and lasted until approximately 0700 h. The measurements were made with the EquiWatch System (Itin+Hoch GmbH,

TABLE 1 The description and test feed arrangement of test horses

Horse no.	Breed	Age	Estimated body weight (kg)	Test feed order (run1, run2)
1	Quarter Horse	16	440	Hay, cubes
2	Quarter Horse	11	509	Cube, hay
3	Quarter Horse	15	544	Hay, cube
4	Paint Horse	12	506	Cube, hay
5	Irish Tinker	9	498	Cubes, hay
6	Quarter Horse	4	473	Hay, cubes

TABLE 2 Chemical composition of hay and cube (mean ± SD[a])

Item	Hay	Alfalfa-mixed meadow grass cubes
Dry matter (%)	91.62 ± 0.02	86.10 ± 0.02
Organic matter (% of DM)	94.80 ± 0.11	87.80 ± 0.32
Crude protein (% of DM)	8.42 ± 0.48	20.64 ± 0.32
Ash (% of DM)	5.21 ± 0.11	12.21 ± 0.32
Neutral detergent fibre (% of DM)	75.53 ± 0.52	49.09 ± 0.24
Acid detergent fibre (% of DM)	45.92 ± 0.66	39.95 ± 0.66
Crude fat (% of DM)	0.95 ± 0.02	1.59 ± 0.05
Non-fibre carbohydrates (% of DM)	9.90 ± 1.13	16.48 ± 0.83

Note: Non-fibre carbohydrates = 100 − (crude ash + crude protein + crude fat + neutral detergent fibre).
[a]Analysed in duplicate.

Liestal, Switzerland), which is a halter equipped with two sensors: a pressure sensor (primary) and a triaxial accelerometer (secondary) embedded into a noseband of the halter. The EquiWatch system was validated for its ability to generate data necessary for characterizing feeding behaviour in horses (Weinert et al., 2020). Halters were monitored through the software RumiWatch Manager, and the data were downloaded from the data logger on the halter and subsequently processed by the RumiWatch Converter software into summaries (Microsoft Excel format) of parameters at chosen intervals. The halter monitors and records eating, chewing, drinking and other activities during feeding, including the head movements of the animal (Werner et al., 2016). Parameters included EATUPTIME (eating time while keeping the head up), EATDOWNTIME (eating time while keeping the head down), EATUPCHEWS (number of eating chews while keeping the head up) and EATDOWNCHEWS (number of eating chews while keeping the head down), the system algorithms of which are explained in a previous publication (Weinert et al., 2020). In addition, there were HEADACTIVITYINDEX (head movement index based on accelerometer data), ACTIVITYCHANGES (number of activity changes between registered activities) and ambient (min, max and average) temperatures included in the present study. Following the validation (Weinert et al., 2020), data from EATUPCHEWS and EATDOWNCHEWS were summed as total chews and used in the present study. Accordingly, total eating time (the sum of EATUPTIME and EATDOWNTIME) was considered.

2.4 | Measuring the dust concentration

Measurements of dust particles during feeding were done using an instrument (Figure S1) consisting of a sampling pump type Gilian 10i (Sensidyne, St. Petersburg, USA), which was installed on the back of the measuring instrument holder together with the data logger DALO (Hund Wetzlar, Helmut Hund GmbH, Wetzlar, Germany). The gravimetric–photometric measuring instrument (Respicon 2 TM, Hund Wetzlar, Helmut Hund GmbH, Wetzlar, Germany) was fixed shock-protected on the front side of the measuring instrument holder and connected to the sampling pump with a coated tube. The pump continuously took in the air over a defined period through the measuring device (Respicon 2 TM), which was divided into three stages, each containing filter. Consequently, three different dust fractions (DIN EN 481, 1993) were collected in the test box, including inhalable (aerodynamic diameter size <100 μm), thoracic (<10 μm) and alveolar permeable (<4 μm) fractions during the test feeding period. After weighing the filters and determining the deposited dust masses, the curve of the concentrations of the inhalable, thoracic and alveolar permeable fractions were calculated over the measurement period. The individual stage of the measuring device was equipped with a scattered light photometer, which permits time-resolved concentration determinations after gravimetric calibration according to the manufacturer. The gravimetric evaluation of the dust fractions on the filters was performed by the accredited laboratory of IC Consulenten Ziviltechniker GesmbH, Vienna.

With a focus on the determination of feed-related dust concentrations, the measuring instrument was installed to allow the sampling in the direct area near the mouth and nose during feed intake. The measurements were carried out in a well-ventilated stable building, and all stable work took place in the morning to minimize a level of exposure to airborne dust. Per test feed, a total measurement period was done in one equivalent horse over four consecutive nights (approx. 40 h).

2.5 | Data management and statistical analysis

There were two data sets for the analyses of chewing activity, both following a crossover design (Cheng et al., 2005). The first data set was for the whole period (overnight) summary data of all horses in both experimental periods. The data were analysed according to test the fixed effects of sequence experimental period and test feed with the random effect of horses nested within the sequence. Next, to evaluate changes associated with time of feeding, data on feeding and chewing behaviour were processed into 1-h intervals starting from their time of feed provision, prior to statistical analysis. The statistical model was the same as the overnight summary plus the fixed effect of duration after feed provision and its feed and time interaction. Interval measurements in each horse were treated as repeated measures, and autoregressive (1) was the covariance structure.

The dust concentration data were combined results of the gravimetric and photometric data. Only inhalable and thoracic particles were considered because the concentrations of alveolar permeable (respirable) particles calculated from the scattered light measurement were below the detection limit of 5 μg/m^3. The dust data were obtained every 5 s. Any occurring drift from the calibrated zero-point of the measuring device was corrected, as recommended by the producer. Subsequently, the data were converted into hourly averages to be consistent with the data presentation of the chewing activity. To account for pre-existing dust loads in the barn, which were assumed to be at fifth percentile concentrations, the fifth percentile value of the respective hour was subtracted from the hourly mean value. The resulting data were used for statistical analysis. The statistical model included fixed effects of test feed, duration relative to the time of feed provision and their interaction and the random effect of the measuring night. Measuring hours in each night were treated as repeated measures with autoregressive (1) as the covariance structure. All statistical analyses were carried out using the MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC, USA). Degrees of freedom were estimated based on the method of Kenward–Roger. Comparisons among least squares means were done following the default PDIFF option (*t*-test) of SAS. The significance level was set at 5%.

3 | RESULTS

3.1 | Feeding duration and chewing activity

The summary of eating time and chew counts is shown in Table 3. On average, the horses spent more than 1 h shorter time per night eating the cubed forage ($p = 0.044$) and had a lower number of total chews ($p = 0.037$) compared to hay. The same comparison was observed on both variables as per kg intake of test feed (as fed basis). When compared per kg dry matter (DM) intake the analysis revealed a trend for less activity when eating cubes ($p = 0.102$ and 0.088 for eating time and total chews, respectively). No difference between hay and cube in the rate of total chews per min of eating time with a rate of 72.6 and 70.6 chews per minute for hay and cube, respectively.

Eating and chewing activity decreased over time (Figure 1). Overall, on an hourly basis, horses spent more time eating and chewing the hay compared to the cube, and there was no interaction between test feed and time of feeding (Table 3). The horses spent the longest eating time at 50 and 40 min in the first hour of feeding hay and cube, respectively, that rapidly declined thereafter (Figure 1). After 7 h, little chewing activity was observed in the cube-fed group, whereas more fluctuation was observed in the hay-fed horses (Figure 1), though the differences did not reach significance. Variables related to drinking and head activity were not different between test feed groups (Table 3). Time spent on other activities and the number of activity changes were higher in cube compared to hay.

3.2 | Dust concentration

The summary and descriptive statistics of raw data (without adjustments with the fifth percentile values) of mass concentrations (mg/m^3) of inhalable and thoracic dust are shown in Table 4. Alfalfa cube feeding led to higher (minimum, mean, median and maximum) concentrations of inhalable particles compared to hay feeding. The maximum concentration of inhalable particles reached 11.99 mg/m^3 for the alfalfa cube feeding compared to 4.87 mg/m^3 for the hay feeding. Figure 2 shows the adjusted air concentrations (subtraction with the fifth percentile value) of inhalable particles throughout the measurement. The alfalfa cube feeding led to higher concentrations of inhalable particles at all hours of measurement (test feed effect, $p < 0.001$). The biggest gap was detected in the first hour after feed provision (Figure 2). The concentrations dropped and remained stable after 2 h of feeding. The same time-dependent change was found for the thoracic dust concentration (Figure 3). Here, the concentrations during the first hour after feed provision were higher with the hay feeding as compared to the alfalfa cube feeding ($p < 0.05$). The overall effect of the test feed was not found for the concentration of thoracic dust ($p = 0.258$), and there was no interaction between feed and duration relative to the time of feed provision ($p = 0.160$).

4 | DISCUSSION

Studies have investigated eating and chewing in horses using different techniques, including electromyography (Brüssow, 2006), optical motion capture (Bonin et al., 2007) and a quartz crystal force sensor (Staszyk et al., 2006), which focus on jaw movements and chew cycles, and telemetry system-based system with collar- and sensor-embedded halter (Weinert et al., 2020; Meyer et al., 1975; Berger et al., 1999; Werner et al., 2016) that also registers frequency and the duration of feeding behaviour. In the present study, the halter used was based on the same technique that was validated in stall-kept horses consuming small meals of concentrate, hay or silage (Werner et al., 2016) and in horses grazing on pasture (Weinert et al., 2020). Overall, the horses spent, on average, 61 min/kg hay DM intake and at a rate of 25.6 min of eating/h and had 72.6 chews/min, which were in good agreement with the ranges reported for hay in these previous studies using the EquiWatch system. Shorter eating times around 39–50 min/kg DM are also reported in the literature (Brüssow, 2006; Meyer et al., 1975; Fritz, 2013; Zeitler-Feicht, 2008), whereas a similar value (74 min/kg hay) to ours was observed in small Icelandic horses (Mixa, 2009). Variations in feeding time are to be expected depending on various factors, such as forage quality, feeding management as well as horse age.

TABLE 3 Chewing activity and ambient temperature following feeding 5 kg (as fed basis) of hay or alfalfa–meadow grass cubes

					p value		
Variables	**Hay**	**SE**	**Cube**	**SE**	**Feed**	**Time**	**Interaction**
Overnight summary							
Total eating time (min)	280	19.9	213	19.9	0.044	–	–
Total eating time (min/kg feed, as fed basis)	56.0	4.0	42.6	4.0	0.044		
Total eating time (min/kg feed DM)	61.1	4.5	49.4	4.5	0.102		
Total chews (*n*)	20,299	1453	15,110	1453	0.037	–	–
Total chews (*n*/kg feed, as fed basis)	4050	291	3022	291	0.037		
Total chews (*n*/kg feed DM)	4420	332	3510	332	0.088	–	–
Total chews (*n*/min)[a]	72.6	1.78	70.6	1.78	0.196	–	–
Hourly data							
Eat time (min/h)	25.55	1.70	19.53	1.63	0.011	<0.001	0.650
Chews (*n*/h)	1844	127	1388	122	0.011	<0.001	0.591
Other activity time (min/h)	34.37	1.70	40.28	1.64	0.013	<0.001	0.634
Drinking time (min/h)	0.11	0.07	0.16	0.07	0.474	0.298	0.237
Drinking gulps (*n*/h)	1.51	0.92	2.09	0.90	0.512	0.229	0.412
Head activity index	54.57	4.48	51.49	4.42	0.382	<0.001	0.160
Number of activity changes (*n*/h)	7.08	0.72	9.34	0.69	0.027	0.075	0.781
Ambient temperature (°C)							
Average	17.30	0.63	15.43	0.63	0.068	0.090	0.556
Min	16.36	0.61	14.14	0.60	0.024	0.087	0.235
Max	18.32	0.72	16.81	0.71	0.119	0.462	0.485

Abbreviation: SE, standard error.
[a]Total chews/total eat time.

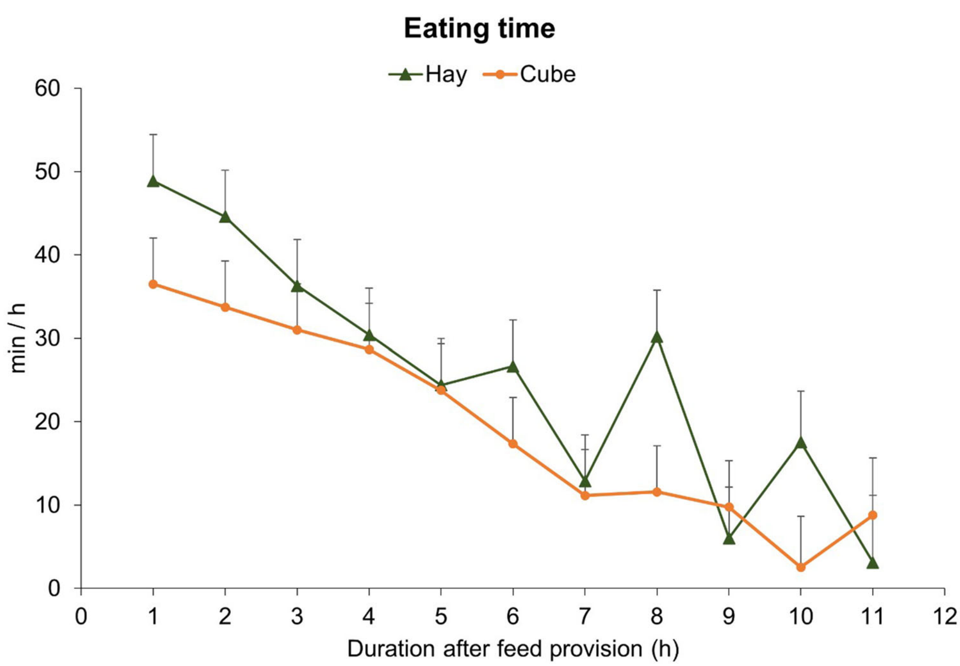

FIGURE 1 Eating time observed overnight following the provision of hay or alfalfa–meadow grass cubes ($n = 6$ per test feed). Feed was provided once at the beginning of the test period. Error bars represent standard errors of the mean. The *p* values of fixed factors are shown in Table 3.

TABLE 4 Descriptive statistics of the raw data on the dust concentrations (mg/m^3) in response to test feed measured every 5 s for four consecutive nights

Item	Test feed[a]	Number of data entires	Mean ± SD	Min	Max	Median
Inhalable particles (<100 μm)	Hay	28,088	0.1945 ± 0.1276	0.0021	4.8743	0.2232
	Cube	28,075	0.4600 ± 0.3669	0.0062	11.9856	0.4004
Thoracic particles (<10 μm)	Hay	28,081	0.0515 ± 0.0762	0.0009	2.9441	0.0337
	Cube	28,075	0.0490 ± 0.0548	0.0003	1.7585	0.0360

[a]Horses received either 5 kg (as fed basis) of hay or cube made from alfalfa–meadow grass mix.

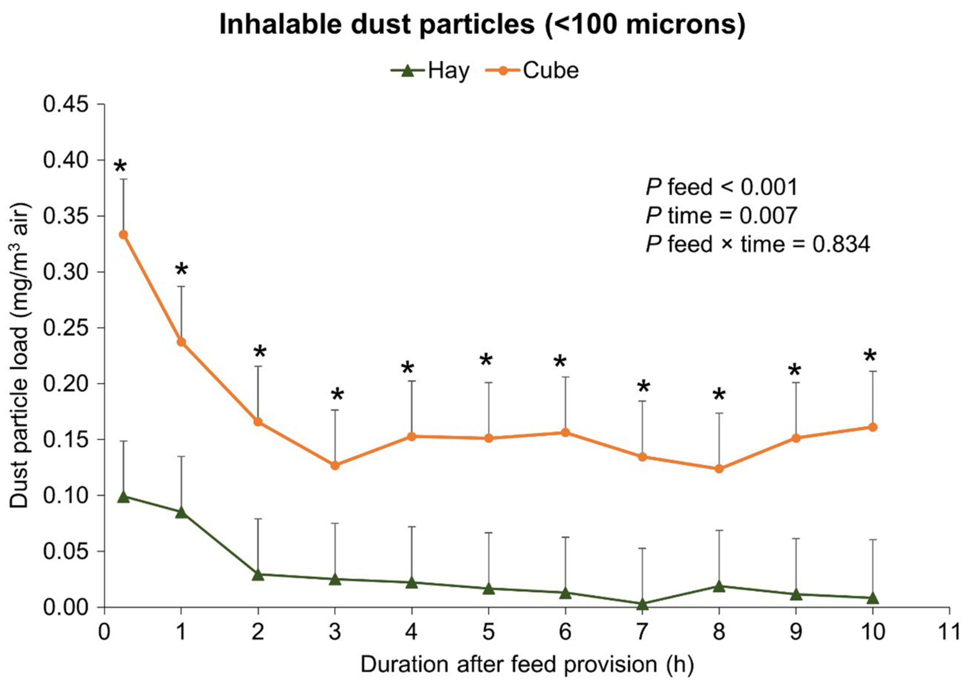

FIGURE 2 Air concentrations of inhalable dust particles in the feeding area when the horse was offered hay or alfalfa–meadow grass cube. The duration of 0 h corresponds to time of feed provision. Data were measured every 5 s for four consecutive nights per test feed. The mean of entries of the hour (e.g. 20:00:01–20:59:56) were adjusted with the corresponding fifth percentile value to account for the pre-existing dust concentration in the barn. Error bars represent standard errors of the mean. Asterisk indicates the differences of values between both test feeds at a given duration ($p < 0.05$). The dust concentrations of the first hour significantly differ from the concentrations detected in later hours ($p < 0.05$).

Smaller horses, as in our study, have relatively smaller chewing surfaces (Zeitler-Feicht, 2008). Meyer et al. (1975) described that ponies need about twice as long for the intake of 1 kg hay compared to large horses.

Although there are investigations for pelleted feed indicating their strong effect on decreased feeding time and chewing behaviour as summarized in a review (Ellis, 2010), there is no such information in the literature for forage pellets, cubes or similar products in horses. We showed that feeding alfalfa–meadow grass cube decreased eating time and number of chews, as a total value and as a rate per hour, compared to hay when these forages were offered overnight for 5 kg (as fed basis) per animal. The lower chewing during cube feeding could be due to the lower content of structural carbohydrates and shorter particle length compared to the long-stem hay. Accordingly, the forage cube had an neutral detergent fibre content of 49.1%, whereas hay has a content of 75.5% of DM. The content of 49.1% can be considered normal for alfalfa and hay harvested at an early maturity stage, whereas 75.5% is indicative of a first-cut hay, harvested at a very late stage of maturity. The reduction is still less dramatic compared to the effect found in pelleted feed (Ellis, 2010; Elia et al., 2010). Furthermore, it must be underlined that when considering the number of chews per eating time or per kg forage DM intake, both test feeds showed no difference with the 70.6 and 72.6 chews per min or 4420 and 3510 chews per kg DM for hay and cube, respectively. Thus, the driving force for the decreased chewing activity when fed cubed forage was the shorter eating time of the cubes, which was on average more than 1 h and likely more feed intake per bite. This might be directly associated with the form of cube vs. long and fibrous strands of long-stem hay with the latter requiring longer chewing time for bolus formation. The longer time spent eating and longer chewing periods are advantageous for horses with decreased energy requirements such as those in maintenance or

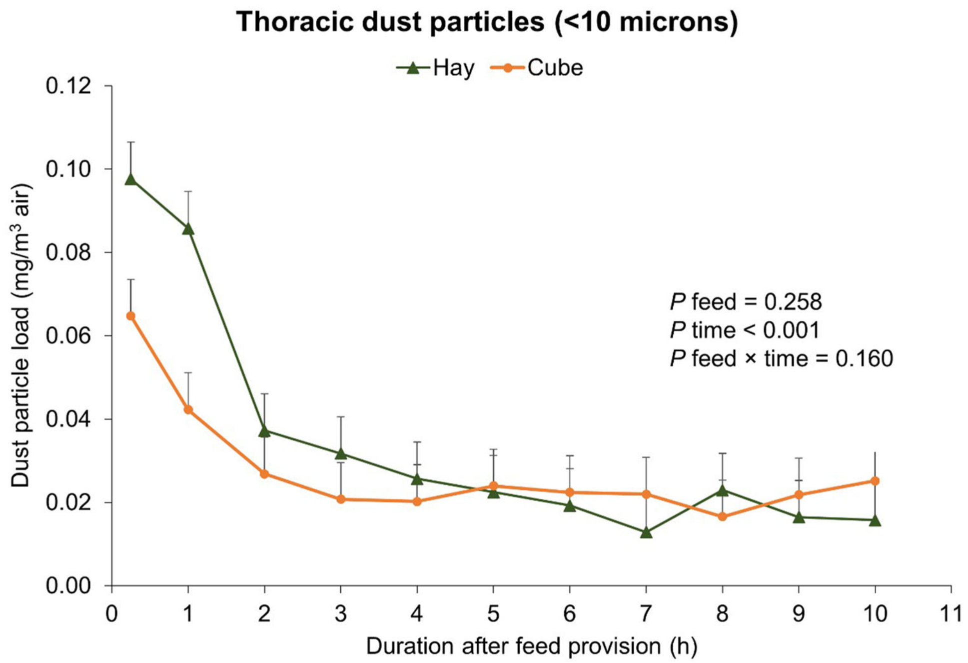

FIGURE 3 Loads of thoracic dust particles in the feeding area when the horse was offered hay or alfalfa–meadow grass cube. The duration of 0 h corresponds to time of feed provision. Data were measured every 5 s for four consecutive nights per test feed. The mean of entries of the hour (e.g. 20:00:01–20:59:56) were adjusted with the corresponding fifth percentile value to account for the pre-existing dust concentration in the barn. Error bars represent standard errors of the mean. Asterisk indicates the differences of values between both test feeds at a given duration ($p < 0.05$). The dust concentrations of the first hour significantly differ from the concentrations detected in later hours ($p < 0.05$).

light work level and also to prevent choke. Data in dairy cattle showed that cubed alfalfa decreased eating time because of decreased DM intake rather than increased ease of ingestive mastication; nevertheless, cubed alfalfa strikingly decreased rumination compared to alfalfa hay (Beauchemin et al., 1997), thereby underlining the significance of forage particle size for chewing behaviour. Mastication stimulates saliva production that buffers the gastric contents, which would promote intra-gastric bacterial fermentation activity (Merrit & Julliand, 2013). Long chewing time is also needed to meet the horse's natural feeding behaviour (GfE, 2014). Despite the decrease in eating time and the number of chews, the values found with the cube in the present work yet remained within the range of 40–80 chews per min, depending on the feed, or 3000–3500 chews per 1 kg roughage reported in the literature (Zeitler-Feicht, 2008). All in all, our data suggest that the cubed alfalfa and meadow grass mix could maintain a normal chewing behaviour of horses in comparison to hay when cubed alfalfa is offered restricted during the night when the horses have ad libitum access to hay during the day. Still, taking into account that the tests were performed with 5 kg feed fed overnight, and the cube-fed horses completed their meal on average 1 h earlier than hay-fed horses, we extrapolated that in the case of 24 h free access to cube feeding, this might have resulted in either higher DM intake and/or decreased eating time of the horse. This could be especially problematic for horses in maintenance and low work level. They have low energy needs, so that higher feed intake of cubes would stimulate overweight or obesity (GfE, 2014). In addition, the high protein and Ca content of alfalfa-based cube when used as a sole forage alternative to hay would be suitable only for some horses with high requirements for such nutrients, such as breeding or growing horses (GfE, 2014), but extra protein would be wasteful for others such as horses at maintenance and low work level. Most importantly, protein overfeeding would increase urea levels in the urine, which also reduces the air quality in stalls by increasing ammonia release (Williams et al., 2011). All being said, more research is needed to evaluate nutritive and environmental effects of alfalfa-based cubes fed ad libitum to horses.

The main sources of airborne particulate matter in horse stables are bedding, feed, hair and skin of the animals and manure (Claußen & Hessel, 2017). Hay is viewed as the main source producing dust in the horse stable (Zeitler et al., 1987; Bartz, 1992), related to organic particles from mould and plant debris. Cubed and pelleted forages are believed to generate less dust than hay. We also expected to see this but it was not confirmed by the results. Although alveolar permeable dust loads (<4 μm) were generally low and thoracic dust loads (<10 μm) were not different between both cube and hay, the inhalable dust (<100 μm) concentration was higher with the cube compared to hay, which was evident in all four nights of measurement. The time of higher loads of dust coincided with the time of eating, and this confirmed that the measurement was related to feeding and not to the stable environment per se. Still, higher concentrations following the cube feeding were also apparent even during low feeding activity. Moreover, the dust measurements of the test feeds were done on different days. Thus, there could be a bias related to the weather, and thus, the ambient (background) dust in the stable may interfere with the true values specific to feeding. By subtracting the inhalable dust concentrations with the plateau concentration, which likely represented the background particulates in the stable, the net concentration of inhalable dust from the cube was still almost three times higher than that of hay during peak feeding time. The dustiness of the cube might be explained by the fact that alfalfa leaves tend to shatter, when too dry. A study observed less dust concentration when keeping horses with shavings and pelleted feed compared to when keeping horses with straw and hay (Woods et al., 1993). A similar outcome was reported for alfalfa pellet compared to good quality grass hay (Vandenput et al., 1997). Cubes are generally dustier than pellets, and our data further suggest that cubes could also result in more dust than hay during feeding. However, this may not raise a concern in terms of respiratory health because it did not affect the small particulate like thoracic and alveolar permeable fractions. Higher concentrations of thoracic and alveolar permeable fractions were shown to be associated with accumulations of trachea mucus and the high numbers of neutrophils (Millerick-May et al., 2015; Millerick et al., 2013). In our study, the concentrations of thoracic fraction observed were generally low (<0.1 mg/m^3) compared to those reported by Millerick-May et al. (2015). The production, storage and presentation of the hay could influence the release of breathable dust (Claußen & Hessel, 2017). On the other hand, our study suggests that when hay is of sound hygienic status and offered in a well-ventilated barn, hay feeding can keep the release of airborne particulate low.

5 | CONCLUSIONS

Compared to the hay, feeding of the cubes made from alfalfa and mixed meadow grass decreased chewing of the horses overnight but primarily due to shorter eating time. The cube feeding also increased dust concentration of inhalable fraction but not that of smaller particles that contribute to the risk of respiratory disease. The effects were, however, considered biologically insignificant when comparing the observed values to the references in literature. Taken together, our data suggest that feeding alfalfa-based cubes overnight generated shorter eating time and less chews than the long hay without major differences in the thoracic dust. Therefore, because of the decreased eating time and chews, alfalfa-based cubes should not be fed as a sole forage source, especially when to be fed without restriction.

AUTHOR CONTRIBUTIONS

Investigation; methodology; writing – original draft: Viktoria Petz. *Data curation; formal analysis; software; validation; visualization; writing – original draft; writing – review and editing*: Ratchaneewan Khiaosa-Ard. *Conceptualization; supervision; writing – review and editing*: Christine Iben. *Conceptualization; funding acquisition; project administration; resources; supervision; writing – review and editing*: Qendrim Zebeli.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon a reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The study was approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna in accordance with the University Guidelines for Good Scientific Practice (protocol number: ETK-38/02/2019 on 02.03.2019).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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