SHORT COMMUNICATION

Palatability of horse diets containing citrus pulp (Citrus sinensis) through the preference test

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Abstract

The aim of this study was to evaluate the acceptance of concentrates containing increasing levels of citrus pulp (7, 14, 21 and 28%) and the most preferred level of inclusion, observing the first action and the palatability using the preference test. Ten healthy mares, averaging 500 kg live weight (LW), were housed in individual pens, where the four experimental concentrates were offered simultaneously, in a linear and continuous feeder for horses, for a period of 15 min. During this period, the first action (smelling or eating), the first choice (first concentrate effectively ingested) and the intake ratio (intake of each concentrate in relation to the total concentrate intake) were observed. Effect of inclusion on first action was observed (P<0.01), once 80% of animals fed promptly the concentrate and only 20% smelled it before the choice. The concentrate with 7% of citrus pulp presented the highest intake ratio (0.4425; P<0.01). For the first choice variable, there was no difference (P>0.05), however the concentrate with 7% was the most preferred (36.67%). Citrus pulp can be used as concentrate feed for horses. Therefore, low levels of inclusion in the concentrate, close to 7%, are recommended when the concentrate is based on corn, wheat bran, soybean meal, with no flavour agents like molasses.

Introduction

Horses are monogastric herbivores, able to supply much of their nutritional requirements by eating grasses (Brandi and Furtado, 2009), but in some physiological stages such as lactation, pregnancy and athlete animals, supplementation with grains or concentrates is needed. Usual concentrates are formulated based on corn, soybean meal and wheat bran, but alternative ingredients such as citrus pulp can be used (Furtado et al., 2011).

Citrus pulp is an energy ingredient (11.92 MJ/kg; NRC, 2007) and has been used in concentrates for horses because it has a high percentage of pectin (20.3%; Rocha Filho, 1998), and in horse nutrition can be considered a super fibre as described by Duren (2000) and Gibbs (2005), because these ingredient energy is near from gains energy. The inclusion of this ingredient in the concentrate may foment a reduction in starch supply in horse meals, being the maximum suggested starch supply of 0.2% per meal (Kienzle et al., 1994). Little is known about the acceptability of this ingredient, being described only by Ott et al. (1979), who found that diets with 30% inclusion of citrus pulp were not accepted by horses. The author suggested that further studies would be needed to determine the maximum level of inclusion of citrus pulp in horse diets.

Like most herbivores, horses select their feed based on visual, odour, taste, availability, texture and variety (Roguet et al., 1998). According to Collery (1974), horses, unlike other herbivores, are highly selective, which is commonly observed when diets are formulated with new ingredients. Goodwin et al. (2005a), evaluating different flavours, suggested that the change in taste may affect foraging and non-foraging behaviour, resulting in the cessation of feed searching.

According to Falkowski et al. (1977) and Edouard et al. (2008), the palatability of plants grazed by livestock is a very complex phenomenon that depends on the participation of phytochemical factors, and also on animal instincts and individual demand. Horses prefer sweeter species, despite the concentration of cellulose. Little is known about the palatability of feeds or complete diets for horses (Goodwin et al., 2005b).

There is a wide divergence in the test protocols for palatability used in horses. According to Carciofi (2008), the palatability test is used to investigate the sensory aspects involved in the feed intake: taste, odour, texture, shape, size, and sensations of chewing and swallowing. For this, two tests may be performed: the acceptance test (employed in order to simulate the intake in farm conditions, when the animal eats only one feed at a time) and the preference test (to confront two or more feeds). This method can be used to compare two or more of commercial diets, changes in the industrial formulation of a same concentrate, or different flavour agents in a same formulation. Thus, feeds are offered simultaneously and the most consumed is classified as more palatable.

The palatability test comprises variables of first choice (first feed actually consumed by the animal) and the rate or ratio of intake (which feed is more consumed) (Carciofi, 2008). For Müller and Udén (2007), the first choice is the feed most consumed during five min and the ratio is considered as feed intake per day. Goodwin et al. (2005b) evaluated the intake rate of the ingredients, the intake of each one, the time of ingestion, and the rejection of some ingredients or the complete rejection for a period of two min. The aim of this study was to evaluate the acceptance of concentrates containing increasing levels of citrus pulp (7, 14, 21 and 28%) and the most preferred level of inclusion, observing the first action and the palatability using the preference test.

Materials and methods

Ten healthy mares, averaging between three and twelve years, with about 500 kg of live weight (LW), were kept grazing Tanzania, Panicum maximum grass (cultivate Tanzania). The animal requirement is about 68,552 MJ/day and 630g CP/day (NRC, 2007). The palatability test based on the preference test (Carciofi, 2008) consisted in simultaneous offering of four concentrates, formulated with different inclusion levels of citrus pulp (Table 1). The concentra-
trates were offered in single continuous linear feeder, located on the rear wall of individual pens. Two boxes were used for observation, where no direct contact between animals was possible but the observer had full vision of feeder. There was no physical division between the different concentrates, with a distance of 30 cm between each. The placement of concentrates in the feeder was randomized everyday in each pen, in order to minimize the animal trend to start selection in a specific point of the feeder. The single feeder was chosen due to animals were used to be fed in the same conditions and observations were easier when animals were eating in it.

The preference test was performed once a week for three consecutive weeks, using the instant focal method (Martin and Bateson, 1986) for a period of 15 min. In this method, the observer look continuously at the animals for 15 min and records all their actions. At these days, mares were removed from pasture and led to the stables; then, they were housed in management area and received 1.0 kg of control concentrated (Table 1), before entering the experimental pens, where the palatability test was carried out. Based on the fact that the mares were kept grazing pasture with forage available during the night before the test, it is advisable that these animals were not hungry before the test. Since they are adapted to receive feed once they arrive at the stables, a control concentrate (with no citrus pulp) was offered. The experimental concentrates were formulated with increasing levels of citrus pulp (Table 1).

All diets were formulate to have protein as near the possible to 12% and 14.64 MJ DE/kg, changes in the proportions of ingredients were necessary (Table 2). The experimental concentrates showed the same appearance and texture, but the odour of the concentrates with higher levels of citrus pulp was more pronounced. The palatability test was divided into two phases: the first choice (first concentrate effectively ingested by the animal) and intake rate (proportional intake of each of the experimental concentrates during 15 min). Besides these evaluations, observations were taken about the first action, being described as the first contact established by the animal and the diet, essential result to check the immediate rejection. Behavioural variables were analyzed by frequency and classified as the first action (one = smelled; two = eat) and as the first choice (according to the experimental concentrates). To determine the intake ratio (IR), 1.0 kg of each concentrate was offered randomly displayed, and 15 min later, the mares were removed from the stalls and the leftovers were measured. The difference between amounts was assumed as intake. The intake ratio was calculated as the proportion of intake of a specific concentrate in relation to the total intake of all concentrates. For a few number of observations (20-30), IR values higher than 0.60 determine the preferred feed, when using two feeds (Carciofi, 2008). Adjusting this value for testing with four feeds, the IR should be higher than 0.30 to determine the preferred concentrate. Adjustments of observed frequencies for first choice and for the first action, along the experimental period, used the Chi-square procedure, considering the hypothesis that all treatments (and choices) occurred with equal probabilities.

Intake ratios observed during period were evaluated using a mixed model, with repeated measures in time that included treatments as fixed effects and random effects of animal. The Student t test procedure was used to compare means. Regression analysis was also used to evaluate the effect of inclusion of citrus pulp on the intake ratio. All tests were performed using the Statistical Analysis System, version 9.1 (SAS, 2001).

Table 1. Chemical composition of experimental concentrates with increasing levels of citrus pulp supplied to horses.

| Ingredient                  | Control | 7% | 14% | 21% | 28% |
|-----------------------------|---------|----|-----|-----|-----|
| Dry matter, %               | 89.24   | 90.01 | 90.68 | 90.41 | 90.8 |
| Ash, % DM                   | 4.33    | 4.38 | 4.77 | 5.15 | 5.53 |
| Crude protein, % DM         | 15.27   | 15.72 | 12.62 | 12.86 | 12.2 |
| Crude fibre, % DM           | 5.02    | 5.78 | 5.8  | 6.05 | 6.97 |
| Ether extract, % DM         | 3.76    | 3.89 | 3.5  | 2.92 | 2.78 |
| Nitrogen free extract, % DM | 70.96   | 70.23 | 73.31 | 73.02 | 72.4 |
| Neutral-detergent fibre, % DM | 15.92 | 15.86 | 15.62 | 15.47 | 16.44 |
| Acid-detergent fibre, % DM  | 12.43   | 12.89 | 12.87 | 12.55 | 12.7 |
| Non-fibrous carbohydrates, % DM | 60.72 | 60.15 | 63.49 | 63.6 | 63 |
| Soluble carbohydrate, % DM  | 3.1     | 2.88 | 4.89 | 5.91 | 6.19 |
| Hemicellulose, % DM         | 3.49    | 2.97 | 2.75 | 2.92 | 3.74 |
| Digestible energy, MJ/kg    | 14.67   | 14.69 | 14.46 | 14.21 | 13.94 |

Table 2. Formulation of experimental concentrates with increasing levels of citrus pulp supplied to horses.

| Ingredients                  | Control | 7% | 14% | 21% | 28% |
|-----------------------------|---------|----|-----|-----|-----|
| Corn                        | 78.57   | 73.04 | 67.36 | 61.12 | 54.88 |
| Soybean meal                | 10.00   | 10.00 | 10.15 | 10.90 | 11.64 |
| Wheat bran                  | 07.28   | 05.81 | 04.34 | 02.83 | 01.32 |
| Citrus pulp                 | 00.00   | 07.00 | 14.00 | 21.00 | 28.00 |
| Mineral premix a             | 02.00   | 02.00 | 02.00 | 02.00 | 02.00 |
| Salt                        | 01.00   | 01.00 | 01.00 | 01.00 | 01.00 |
| Limestone                   | 00.95   | 00.95 | 00.95 | 00.95 | 00.95 |
| Dicalcium phosphate         | 00.20   | 00.20 | 00.20 | 00.20 | 00.20 |

[a]Supplement of vitamins and minerals for horses at all stages of production and reproduction. Security levels, per kilogram: manganese monoxide, 11,400.00 mg; ethoxyquin, 25.00 mg; calcium iodate, 26.00 mg; sodium selenite, 48.00 mg; vitamin B6, 2500.00 μg; calcium pantothenate, 1500.00 mg; biotin, 20.00 mg; vitamin K3, 704.00 mg; choline chloride, 12,836.00 mg; vitamin E, 10,000.00 U; vitamin A, 1,500,000.00 U; vitamin Kc, 240.00 mg; vitamin D3, 150,000.00 U/kg; iron sulfate, 21,700.00 mg; zinc oxide, 25,000.00 mg; copper sulfate, 6250.00 mg.

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Results and discussion

There were differences (P<0.01) for the first action: 20% smelled and 80% of animals ingested promptly the concentrated. There was no difference between treatments (P>0.05) for the first choice. The lowest level of inclusion (7%) was the most preferred (36.67%), followed by 14% (26.67%), 21% (16.67%) and 28% (20.00%) (Table 3).

There was a greater intake of concentrate with 7% inclusion (Table 3), confirmed by regression analysis, with a decrease of 0.01376 (P<0.01) for every 1% of citrus pulp increased in the concentrate (Figure 1). Two statistical approaches (test of means and regression analysis) were used because, as the methodology is rarely used in horses, it was necessary to collect as much information as possible and the concentrate intake is important information for this experiment and also to establish criteria for future experiments. The direct ingestion of most animals (80%) shows that the citrus pulp was not immediately rejected by the animals could be included in concentrates for horses. Ott et al. (1979), testing the inclusion of 30% of citrus pulp in the concentrate, noted only 25% of direct intake by animals, which suggests that the level of inclusion of pulp has an important role on the intake of concentrates. However, unlike the present study; in his study, horses had just one option for intake (30% of inclusion). The act of smelling concentrated before ingest it can be associated to the unknown odour identification, besides the individual effect, associated to the pursuit of preferred feed, the individual ability to select and intake of preferred feed.

The individuality of horses is a well discussed topic (Bonde and Goodwin, 1999; Goodwin et al., 2005a, 2005b; Ott et al., 1979). The high selectivity (Collery, 1974) can be a factor of great interference on individuality. Edouard et al. (2008) mention that feed intake can be influenced by the feed chemical composition, but the variations do not show a consistent pattern. Bonde and Goodwin (1999), studying the behaviour of horses to different odours, found that the horses have strong individual effect, preferred the scents of ginger and coriander and took little time to get closer to the odours of orange oil and lemon fragrance, in addition to spend little time smelling these scents.

The odour is the first and safer contact for the animal, since it does not need to seize the feed to check its taste. It is believed that the horse responds better to the aroma, defined as the perceived organoleptic property via retronasal olfactory organ during the tasting. A simple taste of the feed does not mean the preference, but the curiosity for new feeds, and provide the animal the correlation to the new aroma.

When considering the sensory aspects involved in the feed intake as the flavour, odour, texture, shape, size, sensation of chewing and swallowing (Carciofi, 2008), it was found that the experimental concentrates (Table 1) differed significantly, mainly in the flavour and odour, and the other factors remain practically constant. The increasing level of inclusion of citrus pulp accentuated the smell of this ingredient in the concentrate, which is also reflected in the aroma. Even for the human sense of smell it is noticeable this variation and thus, considering the keener smell sense of the horse, the inclusion of citrus pulp should have been noticed by them. Ott et al. (1979) cited that diets with 30% inclusion of citrus pulp presented quite clear citrus pulp aroma and although pleasant to humans, may have been unpleasant for horses, which refused to concentrate.

The effect of odour on the analysis of the variable first choice was not significant (P>0.05) for treatments. The lowest level of inclusion (7%) was the most preferred (36.67%), followed by 14% (26.67%), 21% (16.67%) and 28% (20.00%) (Table 3). Analyzing these results also revealed the effect of the individual, because among the 10 mares used in the test, only two showed a preference for the concentrate with 28% of inclusion. Studying the choosing behaviour of mares over time, it was found that 60% have the same intake pattern, seeking the same concentrate in at least two of the three observations made for each animal. The concentrate composition may also have influenced the first choice (Table 2). To make concentrates isoeenergetic and isoproteic, there was variation in the proportion of ingredients, mainly in the proportion of corn used in each formulation. As the citrus pulp was included at higher levels, lower percentages of corn were used. As the horse has a great fondness for corn (NRC, 2007), this factor also may have influenced both the first choice, by changing the odour, attributed to the enhancing effect of feeds, and also the intake rate. The decreased level of corn may also have changed the flavour, making the concentrates less sweet, a preference factor for horses (Falkowski et al., 1977), which may have influenced the preference for the concentrate of 7% citrus pulp and the first choice of this inclusion level and also the lower intake of diets containing higher levels of citrus pulp (Figure 1). The preference for the concentrate with 7% of inclusion, observed in the analysis of first choice, was irrefutable by the analysis with the results of the intake ratio, which showed difference (P<0.001) between the concentrates, and the most consumed level was that with inclusion of 7% of citrus pulp. There was a decrease of intake (P<0.01) when citrus pulp was included in the diets (Figure 1). For the other levels, there was no difference (P>0.05), but the most consumed concentrates were respectively 14, 28 and 21%.

Corroborating the above-presented results, when the factor intake ratio and the preference classification index suggested are considerate and adapted by Carciofi (2008) is used, concentrate with 7% of inclusion, is the

![Figure 1. Intake rate of concentrate containing increasing levels of citrus pulp.](image)

| Level of inclusion | First choice, % | Intake ratio | Estimate | SE | DF |
|-------------------|----------------|-------------|----------|----|----|
| 7%                | 36.67          | 0.4432      | 0.0432a  | 0.4432 | 27 |
| 14%               | 26.67          | 0.2715      | 0.4379   | 27 |
| 21%               | 16.67          | 0.1740      | 0.4379   | 27 |
| 28%               | 20.00          | 0.1457      | 0.4432   | 27 |

DF, degree of freedom. *Means different superscripts within column are significantly different (Student t-test, P<0.01).
preferred, presenting $IR=0.44$, emphasizing the preference for this level of inclusion of 7% of citrus pulp in the concentrate. Ott et al. (1979), evaluating the acceptability of diets with oats replaced by different levels of citrus pulp (0, 15 and 30%), also showed similar intake of diets with 15% and without the addition of citrus pulp and there was a partial rejection of the concentrate with 30% citrus pulp (representing 3% of the total offered in 21 days). In a second test, Ott et al. (1979) compared the acceptability of diets containing 30% citrus pulp and citrus pulp-free diets and observed intake of 8.6% of offered concentrate, when that contained 30% citrus pulp and that the offer by extended time (two weeks) did not increase the concentrate intake, similar to data obtained in the present study, where the intake ratio of concentrate with 28% inclusion of citrus pulp had lower intake (0.1457).

In disagreement with the present study, Manzano et al. (1999), evaluating the daily intake of diets with substitution of 0, 7.5 and 15% of corn by citrus pulp in the concentrate, observed greater intake of diets with 15% inclusion of citrus pulp. Again, there were differences in the methodology applied in the test. This author used a complete diet with roughage and concentrates, offered at the same time and together, while in the study of Ott et al. (1979) and in the present work, only concentrate was offered for the intake evaluation. Difference between experiments methods to evaluate palatability and acceptability greatly influence the results. It may be noted that Ott et al. (1979) and Manzano et al. (1999) cited a preference for diets, considering only the intake and refusals, while Müller and Udén (2007) and Goodwin et al. (2005a) applied the test of first choice, however each one considering a protocol, varying mainly in the time of execution and evaluation form.

The present study considered a sum of factors to explain the choice and thus becomes difficult to compare the results with the literature. To better understand the preference and feeding behaviour, the tests must be standardized.

**Conclusions**

The citrus pulp can be used in concentrates for horses. Low levels of inclusion, close to 7%, are recommended when the concentrate is based on corn, wheat bran, soybean meal, with no addition of flavour agents like molasses.

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