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Dried yeast (*Saccharomyces cerevisae*) as a protein source for horses

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A B S T R A C T

The objective of this study was to evaluate the use of dried yeast in the diet of foals when replacing soybean meal to provide 0, 0.25, 0.50, 0.75 and 1.00 of the main protein source. Two trials were performed. Trial one was done to evaluate the influence of yeast on the digestibility of the five diets. Ten foals were kept in metabolic cages and five diets were tested twice. The experiment was repeated a second time, with foals receiving a different diet so that every diet was tested 4 times on a different animal. The concentrate was offered in a level of 2% of the body weight and *Chloris guaiana* hay was offered as roughage in a level of 1% of the body weight. In a second trial thirty 'Brasileiro de Hipismo' foals, aged between 12 and 14 months, were used. They were kept on pastures of *Cynodon dactylon* c.v. Coastcross and *Cynodon* spp. c.v. Tifton 85, and the concentrate was offered twice a day in separate pens at a level of 2% of the body weight. The pastures were of good quality and the pasture availability was high. The experimental design was a 5 × 3 factorial (five levels of yeast and three groups of animals in different pastures), with two replicates/treatment. The foals were weighed and measured every 28 days. Blood samples were collected at the end of the trial. The concentrate intake was measured daily. The pasture intake was measured through estimation of faecal output with chromic oxide and by the amount of acid detergent insoluble ash in the pasture, feed and faecal samples. The mean digestibility of dry matter, organic matter, crude energy and acid detergent fibre did not differ between the diets. A quadratic effect was observed for the digestibility of crude protein and neutral detergent fibre. Increasing the level of yeast in the diets did not have an influence on the average daily weight gain, heart girth gain, withers height gain, but linearly depressed the daily intake of the concentrate. However, the intake of pasture was not influenced by the different diets. The biochemical blood parameters total protein, albumin, globulin, urea, uric acid, creatinine, cholesterol, triglycerides, aspartate aminotransferase and gamma glutamyltransferase were not affected by increasing levels of dried yeast. It was concluded that dried yeast can be used as the only main protein source in the concentrate diets of 12 month old foals.

1. Introduction

It is estimated that 22.3 billion litres of alcohol were produced in Brazil in the 2007/2008 sugar cane harvest (Ministerio do Desenvolvimento, Industria e Comercio, 2008). Considering that it is possible to obtain at least 40 g of dried yeast per litre of alcohol produced (Pinotti, 1997), the potential yeast production for the given period would be in the order of 892,000 tonnes. This potential value is, in fact, ten

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times bigger than the actual value produced in this period for human and animal products (IBGE, 2008), showing there to be considerable scope for increasing the utilization of yeast for animal feeding in Brazil.

The technological changes in yeast production have led to market place yeast having a crude protein content higher than 400 g/kg (Berto, 1997). Since it has a good composition of amino acids, e.g. high levels of lysine, threonine and leucine and, also, high levels of B-complex vitamins, *Saccharomyces cerevisae* may be a satisfactory alternative to soybean meal in animal diets, provided it has no toxic or antinutrient properties.

Several studies have been performed where yeast was added in levels up to 300 g/kg in the concentrate of foals diets, without having an effect on their development (Tosi et al., 1989; Santos, 1992; Whitaker et al., 1995). According to Whitaker et al. (1995), adding 150 or 300 g/kg of yeast to the concentrate of 14 month old foals diets, did not have an influence over the digestibility of dry matter, crude protein, neutral detergent fibre and crude energy. However, Santos (1992) reported an increase in the digestibility of dry matter, crude protein, hemicellulose and neutral detergent fibre in diets with 300 g/kg of yeast in the concentrate, when compared to diets with 200 g/kg of yeast in the concentrate. None of the authors studied metabolic parameters to see if there was an antinutrient effect, and none of them conducted the experiments on pastures.

In rats and fish (*Piaractus mesopotamicus*) respectively, Pacheco (1996) and Padua (1996) reported an antinutrient property when yeast was incorporated in high levels in their diets, leading to a higher mortality rate, slower development, stunted growth and changes in some of the metabolic parameters. These effects were related to the high concentration of nucleic acids in the nitrogen composition of the yeast (8–12% of total nitrogen).

The aim of this study was to evaluate the use of yeast (*S. cerevisae*) in the diets of foals, through the measurement of the growth rate of the animals, the digestibility of the components of the diets, the intake of the concentrate and pasture and the measurement of biochemical blood parameters.

2. Material and methods

Two experiments were performed. Experiment 1 was performed at the Experimental Station of Colina, SP, belonging to the Animal Science Institute of the State of Sao Paulo and aimed to evaluate the digestibility of the diets. A second experiment was performed at the Sao Geraldo Farm, Ipua, SP, Brazil, owned by Haras Agromen. The objectives of this experiment were to evaluate the growth and performance of foals, intake of concentrate and pasture and the possible occurrence of any toxic effects.

Five concentrates were studied, with 0, 0.25, 0.50, 0.75 and 1.00 of the soybean meal protein being replaced by dried yeast protein. The highest level of yeast used in the concentrate was 225 g/kg. The composition of the 5 concentrates used in both experiments, given in g/kg, and their composition in terms of crude protein (CP), digestible energy (DE) and lysine are shown in Table 1. The concentrates were calculated, according to the NRC (1989), to satisfy the requirements of foals at one year of age, with a rapid growth rate and an estimated adult body weight of 600 kg.

Table 1
Composition and feed values (CP, DE, and lysine) of the concentrates with different yeast levels (in dry matter).

	Levels of yeast protein that replace soybean meal protein				
Ingredients (g/kg)	0	0.25	0.50	0.75	1.00
Corn	615	604	593	581	570
Soybean meal	180	135	90	45	0
Dried yeast	0	56	113	169	225
Hay chopped	100	100	100	100	100
Wheat bran	60	60	60	60	60
Mineral supplement	40	40	40	40	40
Vitamin supplement	5	5	5	5	5
Digestible energy (MJ/kg) [a]	13.0	12.0	11.8	11.7	11.6
Crude protein (g/kg) [a]	169	172	167	171	169
Lysine (g/kg) [b]	8.5	8.6	8.7	8.8	8.9

100 g of the mineral supplement provided: 19 g calcium; 7.2 g phosphorus; 6.8 g sodium; 10.5 g chlorine; 2.8 g magnesium; 150 mg zinc; 25 mg copper; 100 mg manganese; 100 mg iron; 1.2 mg cobalt; 2 mg iodine; 0.23 mg selenium, and 0.07 g fluoride.
100 g of the vitamin supplement provided: 160,000 UI vitamin A; 40,000 UI vitamin D_3; 300 UI vitamin E; 64 mg vitamin K_3; 120 mg vitamin B_1; 160 mg vitamin B_2; 50 mg vitamin B_6; 330 mcg vitamin B_{12}; 330 mg pantotenic acid; 2 mg biotin; 600 mg nicotinic acid; 10 mg folic acid; 4 g choline and 20 mg antioxidant (BHT).
[a] Calculated values from NRC (1989).
[b] Values for lysine in soybean meal and yeast were measured, values for other ingredients were taken from NRC (1989).

The composition, in terms of amino acids, of the yeast and soybean meal used in this study is shown in Table 2. The yeast had a 12.6% lower lysine and 10.0% lower threonine level than soybean meal. Yeast is also considered a good source of B vitamins (NRC, 1989).

2.1. Experiment 1 — digestibility trial

Ten 'Brasileiro de Hipismo' foals, aged between 18 and 20 months, were used. 'Brasileiro de Hipismo' is a breed created in Brazil with the aim of producing horses for show

Table 2
Crude protein (g/kg dry matter) and amino acid (g/kg as fed) composition of dried yeast and soybean meal.

Amino acids (g/kg)	Soybean meal	Dried yeast
Lysine	35.0	30.6
Histidine	15.3	10.4
Arginine	41.8	17.5
Aspartic acid	60.0	30.7
Threonine	20.6	18.1
Serine	25.4	16.5
Glutamic acid	91.0	32.8
Proline	22.0	12.6
Glycine	20.1	12.9
Alanine	23.2	19.8
½ Cystine	11.1	6.1
Valine	19.4	28.4
Methionine	9.9	9.4
Isoleucine	26.3	17.5
Leucine	41.8	27.6
Tyrosine	16.2	11.0
Phenylalanine	24.6	13.7
Crude protein (g/kg DM)	450	410

jumping and dressage. The breed originates from the crossing of Thoroughbred mares and German stallions from Hannover, Westfallen and Oldenburg. The mean body weight of the animals was 287 kg. Each of the five diets was offered to four foals at a level of 3% of their BW, twice a day, 2% in the form of concentrate and 1% in the form of roughage. The roughage used was chopped *Chloris gayana* hay.

The animals went through an adaptation period of 15 days, when each foal was kept and fed the experimental diets in a separate pen. A period of total faeces collection of six days followed. During this time the foals were kept in metabolic cages (Stillions and Nelson, 1968). Ten percent less of the concentrate and of the roughage were offered to the foals during the total faeces collection period when compared to the adaptation period, so that no feed would remain left. However, if refusals occurred, they were weighed and a sample was kept for further analysis. The total amount of faeces produced was weighed daily and 50 g/kg was kept in a freezer. Dry matter (DM), crude protein (CP), gross energy (GE), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed in the faeces, feed and refusals samples.

The five diets were randomly allocated to ten foals in two successive three-week experimental periods, whereby each diet was fed to four different foals. The experimental design used was a randomized block design (Snedecor and Cochran, 1967). The data was analysed according to the following linear model:

$$y_{ijk} = m + n_i + b_j + (nb)_{ij} + e_{ijk}$$

where:

y_{ijk} value observed for the coefficient of apparent digestibility of the dry matter, organic matter, crude protein, gross energy, NDF and ADF of the animal k, related to the level i of yeast in the concentrate, in the block j;
m average mean;
n_i effect of the level i of yeast in the concentrate ($i = 1,...5$);
b_j effect of the block j (j = period 1,2) in which the animal is in;
$(nb)_{ij}$ effect of the interaction $n \times b$
e_{ijk} aleatory error related to each observation.

The results were treated to analysis of variance using the post hoc Tukey Test for the comparison of mean values ($P<0.05$). The effects of the yeast level, period and the interaction of both factors were studied. Effects of increasing yeast levels were also subjected to regression analysis.

2.2. Experiment 2 — pasture trial

Thirty 'Brasileiro de Hipismo' foals, twenty males and ten females, aged between 12 and 14 months, were used. Average body weights at the start and end of the experiment were 359 and 457 kg. They were kept on pastures of *C. dactylon* c.v. Coastcross and *Cynodon* spp. c.v. Tifton 85. The ten females were kept on a separate pasture of 400 × 120 m of *C. dactylon* c.v. Coastcross (pasture 5 — 2.0 animals/ha, 1 animal = 359 kg). The males were kept in two smaller pastures of 130 × 150 m of *Cynodon* spp. c.v. Tifton 85 (pastures 1 and 2 — 2.7 and 2.5 animals/ha) and two smaller pastures of 130 × 150 m of *C. dactylon* c.v. Coastcross (pastures 3 and 4 — 3.5 and 3.8 animals/ha), with five animals in each. The concentrate was offered, at a level of 2% of their body weight (BW), twice a day in individual troughs. The foals were restrained by yolks for a period of approximately one hour on each occasion. Any refusals were recorded. On the pasture containing the ten female foals, there were two foals for each of the five concentrates. On the other four pastures, there was one male foal for each of the five diets. All the foals were randomly allocated to the diets and the male foals were randomly allocated to the pastures.

Experiment 2 lasted four months (114 days). The animals were fed the diets, and kept in experimental conditions, for ten days before the start of the experimental period.

The pastures were analysed for DM availability and composition at three times: January, end of February and end of April, with the use of a 0.5 × 0.5 m square. Four to five measures were made randomly per hectare (Euclides et al., 1992). Preliminary observations of the foals grazing suggested a bite at approximately 10 cm height. Accordingly, the samples for chemical analysis were cut 10 cm above ground level. For the measurement of DM mass available, samples were collected near ground level (Gallagher and McMeniman, 1988).

The individual intake of the concentrate was measured every time the animals were fed. A pasture intake trial was done in the month of April. Pelleted chromic oxide (Cr_2O_3) was offered to the twenty male foals, twice a day, at a level of 10 g/day, over twenty days. The aim was to reach a steady state concentration of chromic oxide in the faeces. Faecal samples were collected from the rectum twice a day during the six last days of the trial. Chromic oxide was measured in the faecal samples using the method proposed by Williams et al. (1962), as described by Silva (1981), using atomic absorption spectrometry. Measurements of chromic oxide concentration were used to estimate faecal DM production. Acid Detergent Insoluble Ash (ADIA) was measured as an internal marker in samples of faeces, pasture and feed. ADIA is the residual ash after the determination of ADF and lignin (Silva, 1981 according to Van Soest, 1967). When the ADIA intake in the concentrate feed is known and total ADIA in the faeces is calculated from estimated output of faecal DM, it is possible to estimate intake of DM from pasture. This technique for estimating pasture intake is described by Raymond and Minson (1955).

In the beginning, and after every 28 days, the animals were weighed to the nearest kg, through the use of a weigh-bridge. At the same time measurements of heart girth and withers height were made. Blood samples were collected in March and April from the jugular vein. Total protein, albumin, globulin, urea, uric acid, creatinine, cholesterol, triglycerides, aspartate aminotransferase (AST), creatinine phosphokinase (CK) and gamma glutamyltransferase (γGT) were tested in the serum.

The experimental design was a 5 × 3 factorial (five levels of yeast and three groups of animals on different pastures — male Tifton 85, male Coastcross and female Coastcross) with two replicates per treatment. The results were treated to analysis of variance using the post hoc Tukey Test for the comparison of mean values ($P<0.05$). The effects of the yeast level, group of animal and the interaction of both factors were studied. Effects of increasing yeast levels were also subjected to regression analysis.

A random block design was used for the measurement of pasture intake, with two blocks (pasture), five treatments and two replications. The data was analysed according to the following linear model:

$$y_{ijk} = m + n_i + g_j + (ng)_{ij} + e_{ijk}$$

where:

- y_{ijk} observed value for daily weight gain, monthly heart girth gain, monthly withers height gain, total protein, albumin, globulin, urea, uric acid, creatinine, cholesterol, triglycerides, AST, CK and γGT of the animal k, related to the level i of yeast in the concentrate and the group j of animals;
- m average mean;
- n_i effect of the level i of the factor A (level of yeast in the concentrate) (i = 1,...5);
- g_j effect of the level j of the factor B (group of animals) (j = 1,...3);
- $(ng)_{ij}$ effect of the interaction A × B;
- e_{ijk} aleatory error related to each observation.

The results were treated to analysis of variance using the post hoc Tukey Test for the comparison of mean values ($P<0.05$). The effects of the yeast level, pasture and the interaction of both factors were studied. Effects of increasing yeast levels were also subjected to regression analysis. The SAS (1992) statistical program was used to make the analysis.

The biochemical analysis of serum was performed in the laboratory of the Department of Animal Physiology and Morphology, FCAVJ-UNESP, Jaboticabal, SP, Brazil, with the use of specific kits (Labtest Diagnostica S.A., Av. Paulo Ferreira da Costa 600, Vista Alegre, Lagoa Santa, Minas Gerais, Brazil, 33400-000) and the results were obtained using a spectrophotometer. The methods used for each test were: total protein — biuret, albumin — bromocresol green, urea — urease, uric acid — enzimatic according to Trinder (1969), creatinine — Labtest, cholesterol — enzimatic according to Trinder (1969), triglycerides — enzimatic according to Trinder (1969), aspartate aminotransferase (AST) — kinetic UV, creatinine phosphokinase (CK) — modified Okinaka et al. (1961) and gamma glutamyltransferase (γGT) — modified Szasz (1969). The analysis of DM, CP, ash, NDF, ADF, and GE were carried out in the Laboratory of Animal Nutrition, FCAVJ-UNESP, Jaboticabal, SP and Animal Science Experimental Station of Colina, Colina, SP, Brazil. DM was measured by drying the samples at 100 °C for 24 h, CP was measured using the Kjeldahl method, GE was measured by bomb calorimetry and NDF and ADF were measured using the method proposed by Van Soest (1967), all methods were described by Silva (1981). The analysis of amino acids in the yeast and soybean meal was done in the Laboratory of Protein Chemistry, USP, Ribeirao Preto, Brazil.

3. Results

3.1. Digestibility trial

On most occasions the foals consumed all or more than 90% of the experimental diet. One foal refused significant amount of food in one period. Analysis of variance was performed with and without this animal. Both analyses gave similar results.

Measurements of digestibility from the mixed concentrate: forage diets are shown in Table 3. Analysis of variance revealed no significant effects due to diet, period or interaction of diet and period for any of the measured values. However, regression analysis revealed a significant quadratic effect of replacing soybean meal with yeast on CP digestibility (Crude Protein digestibility = 79.71 − 0.3451 (yeast level in the concentrate) + 0.003 (yeast level in the concentrate)^2, $R^2 = 0.2955$) and NDF digestibility (Neutral Detergent Fibre digestibility = 58.49 − 0.5891 (yeast level in the concentrate) + 0.0052 (yeast level in the concentrate)^2, $R^2 = 0.306$) (Fig. 1).

3.2. Pasture trial

The mean pasture availability, during the four months was 725, 551, 241, 293 and 584 kg DM/100 kg BW in the 1st, 2nd, 3rd, 4th and 5th pastures respectively. The male foals that were held on pastures three and four were transferred to a 6th pasture in February, because of lower availability of DM of these pastures. This new pasture was also formed with *C. dactylon* c.v. Coastcross and had a pasture mass availability of 600 kg DM/100 kg BW. These values indicate that the forage offered was higher than the pasture intake of all the animals throughout the 4 month experimental time.

The mean CP, NDF and ADF values in dry matter for the *Cynodon* spp. c.v. Tifton 85 and *C. dactylon* c.v. Coastcross pastures were: 130 g/kg (108–154), 779 g/kg (761–797) and 417 g/kg (405–428) and 130 g/kg (101–144), 794 g/kg (761–835) and 412 g/kg (372–470), respectively. When testing the same pasture Mandebvu et al. (1999) reported higher CP and lower NDF and ADF levels in dry matter for Tifton 85 and Coastal Bermudagrass, when harvested between 2 and 7 weeks of growth. However, analysing the same forages with 4 weeks of growth Eckert et al. (2010) reported similar values of NDF and ADF and lower values of CP in the DM than the present study.

The increase in yeast levels in the diets had a significant negative, linear, but small effect on the intake of the concentrates (Intake of concentrate = 5.0943 – 0.0083 (yeast level in concentrate), $R^2 = 0.2187$) (Fig. 1). The mean concentrate intake was of 4.68 kg DM/day.

Table 3
Digestibility of dry matter, organic matter, crude protein, gross energy, neutral detergent fibre (NDF) and acid detergent fibre (ADF) of 5 diets containing increasing levels of dried yeast.

	Levels of yeast protein that replace soybean meal protein					Mean	c.v.
	0	0.25	0.50	0.75	1.00		
Dry matter	0.728	0.632	0.647	0.622	0.678	0.662	0.11
Organic matter	0.736	0.643	0.664	0.637	0.689	0.674	0.11
Crude protein	0.800	0.720	0.710	0.700	0.748	0.735	0.09
Gross energy	0.745	0.665	0.670	0.668	0.695	0.688	0.12
NDF	0.598	0.433	0.454	0.430	0.516	0.486	0.23
ADF	0.347	0.250	0.285	0.271	0.368	0.304	0.37

The means do not differ by the Tukey Test ($P<0.05$).

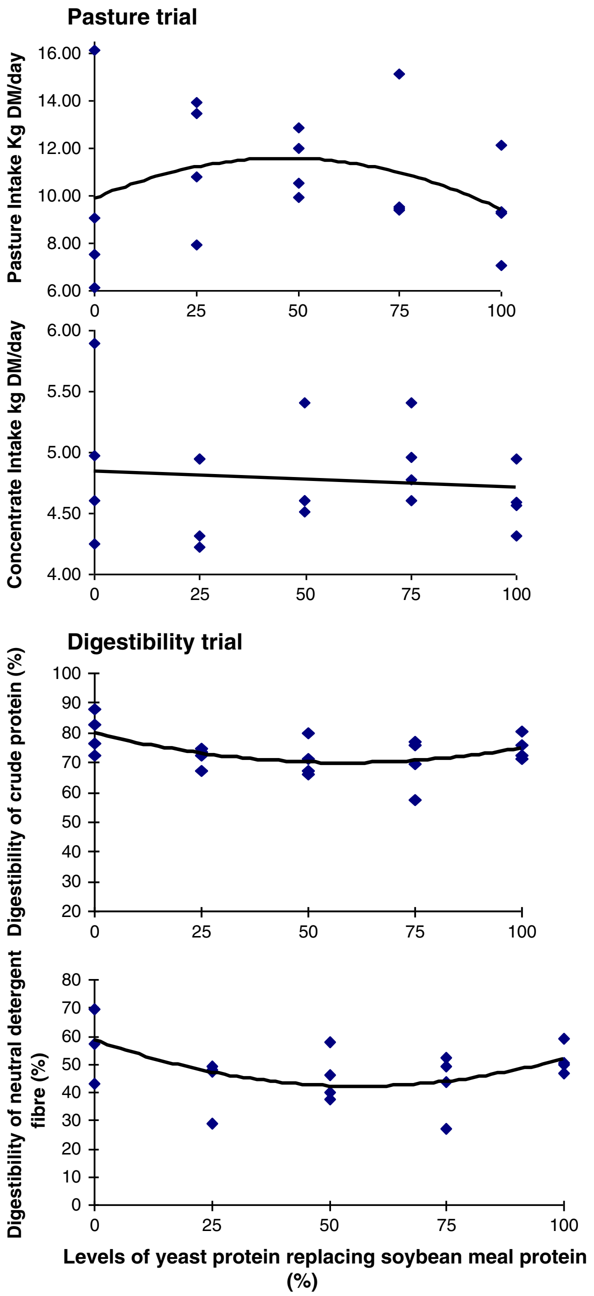

Fig. 1. Pasture intake (kg DM/day), concentrate intake (kg DM/day), digestibility of crude protein and digestibility of neutral detergent fibre of foals fed 5 diets with increasing levels of dried yeast.

Table 4 summarises estimates of total pasture DM intake and the digestibility of the total diet (pasture and concentrate), calculated with the markers Cr_2O_3 and ADIA, to estimate the output of faecal DM and the intake of pasture DM, respectively. The average value for estimated pasture intake was high (10.6 kg DM/day). Neither analysis of variance nor regression analysis revealed any statistically significant effect of yeast substitution on digestibility and estimated pasture intake. It is, however, worthy of note that the estimated pasture intake tended to be higher for the intermediate concentrates (0.25–0.75) (Fig. 1), minimising the effect of the lower digestibility of CP and NDF measured in these concentrates. The intake of *C. dactylon* c.v. Tifton 85 was significantly higher than the intake of *C. dactylon* c.v. Coastcross (11.9 vs. 9.4 kg DM/day).

Mean values for daily weight gain, gain in heart girth (cm/month) and withers height gain (cm/month) over the four measurement periods, are shown in Table 5. The values used for calculation are the mean of the values of the four periods of 28 days. Neither analysis of variance nor regression analysis revealed any statistically significant effect of increasing yeast levels over these parameters.

The values for the biochemical parameters that were tested in the serum, are shown in Table 6. The increase of the yeast level in the concentrate had no significant effect on any of the measured values of total protein, albumin, globulin, urea, uric acid, creatinine, cholesterol, triglycerids, AST, γGT and CK.

4. Discussion

4.1. Digestibility trial

Diet digestibility in horses is affected by the concentrate to forage ration and the diet components (Gibbs et al., 1996), however not the intake level (Martin-Rosset and Dulphy, 1995). Overall organic matter and gross energy digestibility coefficients in the present study were similar to the coefficients reported by Martin-Rosset and Dulphy (1995) in diets with 60% concentrate, the same level to the one offered in the digestibility trial. Whitaker et al. (1995) fed 40% concentrate in their diets (forage to concentrate ratio of 3:2), used alfalfa as forage and similar ingredients in the concentrate as the present study. Those authors found similar digestibility coefficients for the DM and GE however, lower coefficients for CP and NDF, which could have been affected by the concentrate to forage ratio and type of forage used. Martin-Rosset and Dulphy (1995) and Whitaker et al. (1995) used yearlings in their studies. In adult ponies Hintz et al. (1971) obtained higher digestibility

Table 4
Pasture intake and digestibility of dry matter (DM) from two pastures, *Cynodon dactylon* c.v. Coastcross (CC) and *Cynodon* spp. c.v. Tifton 85 (Tifton), of foals fed concentrates with increasing yeast levels.

	Levels of yeast protein that replace soybean meal protein					Tifton	CC	mean	c.v.
	0	0.25	0.50	0.75	1.00				
Pasture intake (kg DM/day)	9.7 [a]	11.5 [a]	11.4 [a]	10.9 [a]	9.5 [a]	11.9 [c]	9.4 [d]	10.6	0.11
Digestibility DM	0.668 [a]	0.690 [a]	0.678 [a]	0.683 [a]	0.670 [a]	0.719 [c]	0.636 [d]	0.678	0.09

[a, b]Means followed by the same letter do not differ by the Tukey Test ($P<0.05$), effect of yeast substitution.
[c, d]Means followed by the same letter do not differ by the Tukey Test ($P<0.05$), effect of pasture.

Table 5
Gains of weight (kg/day), heart girth (cm/month) and withers height (cm/month) of the foals fed concentrates with increasing yeast levels (over 114 days).

Gain	Levels of yeast protein that replace soybean meal protein					mean	c.v.
	0	0.25	0.50	0.75	1.00		
Weight (kg/day)	0.72	0.65	0.69	0.76	0.73	0.71	0.14
Heart girth (cm/month)	2.49	2.47	3.00	3.12	2.46	2.70	0.37
Withers height (cm/month)	2.47	1.76	1.80	2.19	2.29	2.10	0.36

The means do not differ by the Tukey Test ($P<0.05$).

coefficients of the DM and NDF when offering diets with a slightly higher (3:2) and lower (1:4) forage to concentrate ratio than the present study. The digestibility coefficient of CP was higher in diets with more concentrate and similar to the present study in diets with 3:2 forage to concentrate ratio. Similarly to Whitaker et al. (1995) those authors used alfalfa as forage and soybean meal and corn in the concentrate and obtained higher digestibility coefficients in all nutrients. When offering diets with a 2:1 forage to concentrate ratio to adult horses and using hay as forage Alvarenga et al. (1997), Vermorel et al. (1997) and Miraglia et al. (1999) reported higher digestibility coefficients for NDF and ADF and lower digestibility coefficients of DM, OM and CP when compared to the present study. The digestibility coefficient of energy was also lower (Vermorel et al., 1997; Miraglia et al., 1999). There was a tendency for adult horse to better digest the fibrous components of the diet when compared to growing horses. However, the higher intake of forage and the use of different diet components in those studies when compared to the present study could certainly have affected those results making comparisons difficult. More studies are necessary to compare differences between growing and adult horses in their ability to digest different diets.

As illustrated by the quadratic equations (Fig. 1) the digestibility for CP and NDF were higher in the all-soybean meal-diet and the all-yeast-diet than in the intermediate diets. Gibbs et al. (1996) reported a total tract digestibility of soybean meal nitrogen of 92.17%. Similarly to the present study, Santos (1992) reported higher digestibility of DM, CP, NDF and hemicellulose for diets with 300 g/kg yeast in the concentrate, when compared to diets with 200 g/kg yeast in the concentrate. Whitaker et al. (1995) did not find any differences in DM, CP, NDF and CE digestibility in diets with 0, 150 and 300 g/kg of yeast in the concentrate, but their data was not submitted to regression analysis. According to the present study yeast protein can be used as a substitute for soybean meal protein for horses without practical consequence on the digestibility of DM, OM, CP, CE, NDF and ADF. There was no evidence of an antinutrient effect on the digestibility of CP due to the yeast supplementation.

4.2. Pasture trial

The observed weight gain (0.71 kg/day) was similar to the expected weight gain given by the NRC (1989) for 12 month old foals with an estimated adult body weight of 600 kgs (0.60–0.80 kg/day) and higher than the figures given by the NRC (2007) of 0.54 kg/day. Hansen et al. (1987) also obtained a similar daily weight gain (0.69 kg/day) and withers height gain (1.9 cm/month), in foals of the same age, held on a pasture of *C. dactylon* and receiving a supplement of 2.3 kg concentrate/day. However, these authors reported that foals held on the same pasture, but without receiving any concentrate, had the same withers height gain as the supplemented foals, but lower daily weight gain. No impairment of growth was noticed and their supplemented foals had more deposits of fat than the other foals. Grace et al. (2002) reported a daily weight gain of 0.6 kg/day for Thouroughbred yearlings at pasture without supplementation. Graham et al. (1994) fed a concentrate containing 12% CP at a level of 2% of the BW to Thoroughbred and Quarter Horse yearlings. The diets were supplemented with Coastal Bermudagrass hay and with lysine, lysine and threonine or not supplemented with amino acids. Lysine and lysine and threonine supplemented foals had a significantly higher daily weight gains (0.64 and 0.67 kg/day) and girth gains (2.52 and 2.82 cm/month) when compared to amino acid non supplemented foals (0.57 kg/day and 2.42 cm/month). No difference in height gain was observed between the groups (1.27, 1.20 and 1.25 cm/month, respectively). Concentrates in the present experiment were balanced for the CP and lysine level and no difference in weight, heart girth and height gain were

Table 6
Biochemical parameters measured in the serum of foals fed 5 concentrates with increasing levels of yeast.

Parameters	Levels of yeast protein that replace soybean meal protein					Normal values [a]	c.v.
	0	0.25	0.50	0.75	1.00	Min.–max.	
Total protein (g/l)	93.3	81.2	85.6	87.5	85.3	48–79	0.08
Albumin (g/l)	35.2	36.8	39.9	37.9	39.9	21–48	0.15
Globulin (g/l)	58.1	44.4	45.7	49.6	45.3	19–43	0.20
Urea (mmol/l)	19.8	20.7	22.6	21.7	22.8	1.33–24.1	0.27
Uric acid (μmol/l)	0.27	0.27	0.24	0.26	0.25	0.53–0.65	0.26
Creatinine (μmol/l)	108.6	105.1	116.4	119.8	119.9	80–200	0.12
Cholesterol (mmol/l)	2.48	2.49	2.49	2.52	2.47	0.29–4.86	0.13
Triglycerides (mmol/l)	0.32	0.31	0.32	0.47	0.39	0.11–0.97	0.47
Aspartate aminotransferase (UI/l)	23.8	24.1	25.4	25.7	24.6	58–366	0.12
gamma glutamiltransferase (UI/l)	13.9	12.1	14.3	21.4	13.5	0–40	0.55
Creatinine phosphokinase (UI/l)	43.5	11.5	16.2	3.4	16.3	Until 200	2.41

The means do not differ by the Tukey Test ($P<0.05$).
[a] According to Meyer (1989), Kaneko (1989) and Johnston (1991).

observed. The CP content of the concentrate was higher than the concentrate used by Graham et al. (1994) (16% CP) and probably exceeded the requirements of the foals.

The blood values for total protein, globulin and urea were high in all animals, when compared with the results found by Meyer (1989), Kaneko (1989) and Johnston (1991). These high values are probably related to a high intake of CP, caused by the good pasture quality and high levels of concentrate offered. Saastamoinen (1996) reported an increase of 8 to 10% in the values of total protein in serum, in animals that were receiving an excess of protein in their diets and diets that were out of balance with respect to amino acids. An excess protein intake can also lead to an increase in the values of urea in the serum (Fonnesbeck and Symons, 1969; Patterson et al., 1985; Schryver et al., 1987; Saastamoinen, 1996). Serum urea concentration is affected by the rate of synthesis in the liver and the rate of clearance in the kidneys. The rate of synthesis is influenced by the protein in the diet and the protein catabolism (Stockham, 1995).

Uric acid is the product of the metabolism of purinic bases (adenine and guanine), that are part of the nucleic acids. Non primate mammals metabolise uric acid to allantoin, which is then excreted in urine (Pacheco, 1996). The values of uric acid remaining in the normal range and the absence of any difference between the animals receiving the diets with increasing yeast levels is an indication that all of the nucleic acid was metabolised and no accumulation occurred. The normal values of creatinine indicate that the kidneys were functioning normally.

The 2 enzymes measured in the serum, AST and γGT, which are normally encountered in the liver, could indicate in horses damage to the hepatocytes or biliary ducts (Stockham, 1995). Damage could occur if the uric acid was not metabolised and accumulated in the hepatocytes. Curran et al. (1996) measured γGT and AST in healthy horses and horses with subclinical hepatic diseases and found a sensitivity of 0.75 for γGT and 0.58 for AST and a specificity of 0.90 for γGT and 1.00 for AST. Only 1 animal had a value of γGT, slightly, over the normal range (59.3 UI/l). This animal was receiving 169 g/kg of yeast in the concentrate. The other serum parameters measured were in the normal range for this animal and other γGT measurements would have to be taken before concluding that liver damage was occurring. The absence of any difference between the mean values of the animals fed the five diets, and of any regression effect, along with the fact that most of the values were in the normal range, indicate that no liver damage was occurring related to the addition of yeast in the diets. Cholesterol is esterified in the liver and triglycerides are formed in the liver (Bauer et al., 1990) and the values in normal range reiterate that the liver metabolism was functioning normally.

The observed excellent growth rate and the lack of difference in growth and blood biochemistry, once again demonstrates the absence of an antinutrient effect of yeast on growth and liver metabolism, as opposed to that described in rats and fish by Pacheco (1996) and Padua (1996), respectively. In pigs Moreira et al. (1998) and Landel Filho (1991) reported a negative linear effect on the weight gain with increasing levels of yeast in the diet of growing finishing pigs. In the experiment of Landel Filho (1991) the levels of uric acid increased linearly with increasing levels of yeast however, were still in the normal range. Santos (1992) and Whitaker et al. (1995) did not find any difference in the weight gain, withers height and thoracic circumference between foals that were receiving diets with increasing levels of yeast.

Although intake and digestibility were greatest for the all-soybean meal-diet in all of the assessments, satisfactory results were obtained with all diets containing yeast. Indeed the all-yeast-diet (1.00) tended to be superior to the intermediate diets. Concentrate intake at pasture declined slightly with increasing yeast substitution and this was not accompanied by a compensating increase on pasture intake. Nevertheless this did not compromise growth.

The lower intake of the concentrate with higher levels of yeast (169 and 225 g/kg) (Fig. 1) may be related to the fine texture of the yeast. Berto (1997) came to the same conclusion when lower intakes were found when higher levels of yeast were added to pig diets. Tosi et al. (1989), Santos (1992) and Whitaker et al. (1995) did not find any problems related to diet acceptance with different levels of yeast fed to horses.

Estimated mean pasture intake was 10.6 kg DM/day (26 g/kg BW or 116.8 g DM/kg $BW^{0.75}$/day). This intake is within the range given by Pagan (2000) of 1.0 to 2.5% of the BW of forages for yearling horses. Grace et al. (2002) reported an intake of 6.9 kg DM/day for yearling Thoroughbred horses (350 kg) grazing a ryegrass/white clover pasture. The estimated intake in the present experiment is high compared with the published values for true intake (not estimated) of fresh forages in adult light horses (average 500 kg BW) at maintenance (19–22 g/kg BW), by Dulphy et al. (1997). Gallagher and McMeniman (1988) reported a mean dry matter intake at pasture of 9.01 kg/day in the winter and 10.21 kg/day in the spring in mares weighing 500 to 540 kg. Those authors used chromic oxide as external marker to measure intake. Fleurance et al. (2001) measured an intake of 172.5 ± 23.1 g DM/kg $BW^{0.75}$/day and Menard et al. (2002) measured an intake of 144 g DM/kg $BW^{0.75}$/day in adult horses feeding in natural grasslands. The intakes above were considerably higher than those observed in the present experiment. They were measured through total faecal collection and estimating digestibility by the crude protein content of the faeces (Fleurance et al., 2001) and published values (Menard et al., 2002).

The intake and DM digestibility of the Tifton 85 pasture were higher than the Coastcross pasture. However, Edouard et al. (2008) found a compensatory intake with decreasing digestibility of forages in horses. As intake was high in horses in both types of pasture this difference in intake reflected probably more the organoleptic qualities of the forages (ease of prehension, toughness, ease of sorting, and taste), as suggested by Dulphy et al. (1997). Probably the greater availability of the *Cynodon* spp. c.v. Tifton 85 pasture had an influence on the difference in the intake between both of the types of pasture. Fleurance et al. (2009) reported in horses that the bite size increased linearly with the increase in the sward biomass. However, Gallagher (1996) observed that as the bite weight increased in horses the bite rate decreased. Mandebvu et al. (1999) found that Tifton 85 produced more DM, NDF and ADF, and that the digestibility of DM, NDF and ADF in steers was higher when compared to Coastal Bermudagrass. Eckert et al. (2010) did not find differences between the digestibilities of both forages when offered to horses as hay cut in the same period of regrowth (4–5 weeks).

The DM digestibility of the hay of both forages determined by total faecal collection was lower than the digestibility measured in the present study.

Assuming a mean intake of 10.6 kg DM/day of pasture, the mean DE intake of these foals would be 153 MJ/day (102 MJ/day from pasture and 51 MJ/day from concentrate). The DE requirement for maintenance (with increase of 0.25 of maintenance because of activity) and growth of a foal with 410 kg BW and a weight gain of 0.71 kg/day is 130 MJ/day (NRC, 1989). The double marker technique (Cr_2O_3 and ADIA) is inherently liable to overestimate pasture intake since failure to recover 100% Cr_2O_3 in faeces overestimates faecal output (Haenlein et al., 1966). Haenlein et al. (1966), Knapka et al. (1967) and Parkins et al. (1982) have reported a lower recovery of chromic oxide in the faeces, when compared to the complete collection of faeces. Fontes et al. (1996) found a recovery rate of 100% of chromic oxide when faeces were sampled twice daily when compared to total faecal collection. Raymond and Minson (1955) reported a daily fluctuation in the faecal chromic oxide concentration, this fluctuation being linked to the times chromic oxide was fed to the animals and the rate of intake that affected the rate of passage of food. Reasons for a non total recovery of the chromic oxide in the present experiment could have been the time of faeces collection (mornings and afternoons), the way the chromic oxide was offered (pelleted), which could lead to some losses, and the collection of faeces from the rectum (Raymond and Minson, 1955; Haenlein et al., 1966).

Miraglia et al. (1999) discussed the use of indigestible internal markers in field studies with horses. Very good recovery rates were reported for the marker acid insoluble ash (AIA) and no significant differences of the digestibility of DM, OM, energy, CP, ADF and NDF when compared to total faecal collection. Similar results were reported by Bergero et al. (2004) when measuring digestibility by total faecal collection and AIA except for coefficients obtained for CP, which were very variable. Goachet et al. (2009) found that collection time affected the digestibility coefficients when using AIA. The digestibility coefficients were lower with a 3 day collection period than a 4 to 5 day collection periods. Stein et al. (2006) found that the DM intake and the DM digestibility coefficient affected the estimate of digestibility when measured by the markers indigestible cellulose and indigestible acid detergent fibre (iADF). AIA estimates were affected by the DM digestibility coefficient. Only the digestibility coefficients measured by iADF did not differ to the coefficients obtained by total faecal collection. Different authors reported poor recovery of the marker acid detergent lignin (ADL) and lignin and significantly underestimated digestibility coefficients (Miraglia et al., 1999; Araujo et al., 2000a; Goachet et al., 2009). Araujo et al. (2000a) attributed the poor recovery of lignin to the digestion of its different components and the variation in its chemical composition. Araujo et al. (2000a,b) obtained a good recovery of the internal markers AIA (93.7–101.3%) and ADIA (97.7–99.7%) in mixed concentrate and roughage diets and diets made out only of roughage fed to horses. The digestibility coefficients of OM, DM, CP, NDF, ADF and GE measured by AIA and ADIA did not differ to the ones obtained by total faecal collection. The concentration of ADIA found in the pastures in the present study varied between 1.3 and 1.9% in the DM and was almost inexistent in the concentrates (0.02–0.2% in the DM). Araujo et al. (2000a) reported values of 1% of ADIA in the DM of Coastcross hay. In both studies the low concentration of the marker in the feed did not affect its faecal recovery.

Any overestimation of pasture intake is likely to be consistent for all concentrate diets, so these estimates are valid for comparative purposes. Moreover estimated DM mean digestibilities were very similar in Experiment 1 (0.662 in metabolic cage) and Experiment 2 (0.678 on pasture). A systematic tendency to overestimate faecal output (and the pasture intake), when concentrate intake is known, would tend to underestimate overall digestibility of pasture and concentrate. The similarity between estimates of digestibility in Experiments 1 and 2 supports the contention that these estimates of pasture intake do not involve any serious, systematic error.

5. Conclusion

The mean digestibility of dry matter, organic matter, crude energy and acid detergent fibre did not differ between the diets. A quadratic effect was observed for the digestibility of crude protein and neutral detergent fibre. Increasing the level of yeast in the diets did not have an influence on the average daily weight gain, heart girth gain, withers height gain, but linearly depressed the daily intake of the concentrate. However, the intake of pasture was not influenced by the different diets. The biochemical blood parameters were not affected by increasing levels of dried yeast. It was concluded that dried yeast can be used as the only main protein source in the concentrate diets of 12 month old foals.

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