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Effect of Dietary Supplementation of Linseed Oil on Palatability and Nutrient Utilization in Marwari Horses

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ABSTRACT

The present study was aimed to investigate the effect of dietary supplementation of linseed oil on palatability and nutrient utilization in Marwari horses. The feeding trial was conducted at ICAR-National Research Centre on Equine, Equine Production Centre, Bikaner for 60 days. Eight Marwari horses divided randomly into 2 treatment groups of four horses each were adapted to consume experimental diets consisting of groundnut straw as a sole source of roughage in both the groups and concentrate mixture with no linseed oil (Low Fat Concentrate) and with 8% linseed oil/kg (High Fat Concentrate) in T1 and T2 groups, respectively. Dry matter and organic matter intake were significantly lower ($P<0.05$) on linseed oil supplementation in terms of kg/d (9.12±0.03 *vs* 8.95±0.06 for DMI and 8.21±0.03 *vs* 8.03±0.05 for OMI) whereas, the dry matter and organic matter intake in terms of kg/100kg BW and g/kg$W^{0.75}$ demonstrated significantly lower values for horses supplemented with linseed oil only during IV fortnight. The inclusion of linseed oil in the concentrate mixture had no adverse effect on the palatability of diet and it offered a good acceptability by the horses. Linseed oil significantly ($P<0.01$) increased the apparent digestibility of dry matter (64.1 *vs* 60.31%), ether extract (79.7 *vs* 62.4%) and neutral detergent fiber (54.5 vs 48.8%) of the diet. The linseed oil also significantly increased ($P<0.01$) the TDN (670.8 vs 626.3 g per kg) and ME (2.42 vs 2.26 Mcal) content of diet. It can be concluded that dietary supplementation of linseed oil at the rate of 8% in the concentrate mixture of horses is an easy and safe way to increase energy density of diet and at such inclusion rate no negative effect on digestibility and feed intake was observed.

KEYWORDS: Digestibility, Horses, Linseed oil, Palatability

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INTRODUCTION

In working horses to meet an increased demand for dietary energy, high levels of cereal grains are regularly used (Delobel et al., 2008). Although the total intestinal tract digestibility of starch is high in horses, the capacity of starch digestion in the small intestine is limited. So, high intakes of poorly digestible, highly fermentable carbohydrates may exceed the hydrolytic capacity of small intestine. This excessive starch enters in large intestine and leads to altered microbial fermentation in the caecum (Meyer et al., 1993) and may even cause caecal acidosis (Garner et al., 1978) which ultimately leads to depression in fibre utilisation. Owing to this high level of starch associated disturbances, gastrointestinal disorders like colic and other related disorders such as laminitis can also be observed (Robaye et al., 2017). Thus, replacement of cereals with fat is an alternative for increasing the energy density in the diet while reducing the risk of digestive problems related to undigested cereal starch (Lindberg and Palmgren Karlsson, 2001). In addition, when fat is substituted for carbohydrates, there may be a reduction in the heat produced from fermentation in the hindgut (Mc Cann et al., 1987; Potter et al., 1990), due to the change in diet composition as well as due to reduction in dry matter intake.

Dietary fat is becoming more widely used as a concentrated source of energy for horses subjected to intense training and work (Hughes et al., 1995). Substantial research has demonstrated that performance horses get benefited from supplemental fat as a source of energy, and the caloric and extra caloric benefits of feeding fat-supplemented diets in performance of working horses are well documented (Meyers et al., 1989) but researches regarding the effects of fat supplementation on nutrient digestibility are quite limited. It was therefore of interest to evaluate the specific effect of fat on digestibility in non-performing horses. However, because equids have phylogenetically been adapted to have only small amounts of fat in their feed, the tolerance level of dietary fat must be completely analysed. Vegetable oils like soybean oil, corn oil and linseed oil etc., are extensively used in equine industry to increase the energy density of the diet and to provide essential fatty acids although their fatty acid profiles vary. The advantage of linseed oil over other vegetable oils stems from its high content of omega-3 polyunsaturated fatty acids (PUFA), especially alpha-linolenic acid (ALA), of which it is the richest plant source (Pilar et al., 2017). Enrichment of the diet with omega-3 acids, e.g., using linseeds or linseed oil, may help to suppress inflammatory and allergic reactions, improve the blood lipid profile, increase tissue sensitivity to insulin and proved effective in preventing diabetes (Shahidi et al., 2018). Most of the research works on linseed supplementation in equines were geared towards the therapeutic management of equine disorders rather as feed additives (Elghandour et al., 2018). Therefore, a greater understanding and knowledge on utilization of linseed oil in the equines diets may require the concerns over their usage, thus promoting them as feed additives to improve equine health and wellbeing. Therefore, this study aims at comparing the palatability as well as the total tract apparent digestibility of gross nutrients and fibre fractions in horses receiving a concentrate enriched with linseed oil.

MATERIAL AND METHODS

Eight healthy Marwari stallions of uniform conformation, age 4-8 year and body weight 380±50 kg were selected from ICAR-National Research Centre on Equine, Bikaner campus. The horses were distributed randomly in two treatment groups of four each for conducting feeding trial for the period of sixty days. Horses were gradually introduced to diet treatments for 7 days to prevent diet-related health disorders. Each stallion was housed separately in their hygienic and well ventilated sheds having separated feeding and watering system. Prophylactic measures were taken against common parasitic infestations. The experimental feeds were formulated to meet the nutritional requirement of horses according to Indian feeding standards (ICAR, 2013). Taking into account the safe level and previous researches (Delobel et al., 2008; Williams et al., 2017; Bush et al., 2001) the limit of 8% fat was chosen as an approximate value to be added in the experimental diet in the present study. The type of dietary fat selected was linseed oil. The horses were fed diets consisting of groundnut straw as a sole source of roughage and concentrate mixture with no linseed oil (T1 group: Low fat concentrate) and with 8% linseed oil/kg (T2 group: High fat concentrate). The parts composition of ingredients of experimental feeds has been presented in Table 1 and the chemical composition of groundnut straw and experimental concentrate mixture are given in Table 2.

Table 1. Ingredient composition of experimental concentrate mixtures

Feed Ingredient	Low Fat Concentrate	High Fat Concentrate
Gram	10	10
Soybean meal	10	10
Barley	45	37
Wheat bran	32	32
Mineral mixture	3	3
Linseed oil	0	8
Total	100	100

At the end of feeding trial; a digestibility trial of 6 days duration was conducted for estimation of digestibility of the gross nutrients and fibre fractions by conventional total faeces collection method. In addition, the average time expended by the horses to consume 1 kg concentrate mixture was recorded during the six days of digestibility trial and used as palatability index.

Table 2. Chemical composition of groundnut straw and experimental concentrate mixtures (on DM basis)

Nutrient	Groundnut Straw	Low Fat Concentrate	High Fat Concentrate
Dry Matter (%)	89.0	91.3	91.1
Organic Matter (%)	90.7	93.3	93.6
Crude Protein (%)	8.47	15.8	15.6
Ether Extract (%)	1.49	4.13	9.82
Crude Fibre (%)	28.7	18.3	16.9
Nitrogen Free Extract (%)	52.0	54.9	51.2
Total Ash (%)	9.23	6.67	6.35
Neutral Detergent Fibre (%)	49.3	36.1	34.9
Acid Detergent Fibre (%)	30.0	14.8	13.8
Hemicellulose (%)	19.3	21.2	21.0

The feed and faecal samples were analysed for proximate constituents according to AOAC methodology (2016). Moisture, dry matter and total ash of samples were analyzed by standard conventional procedure. Ether extraction and crude fibre of feed and faeces were estimated by using Soxhlet's apparatus and Fibre-E-Tek machine, respectively. Estimation of total nitrogen of feed, residue and faeces were done by standard Kjeldahl's method using KelPlus semi-automatic nitrogen analyzer. The method of Van Soest (1991) was adopted to evaluate the cell wall constituents.

In vivo apparent digestibility coefficients of Dry matter, Organic matter, Crude protein, Crude Fibre, Ether extract, ADF and NDF were calculated as (Nutrient intake-Nutrient outgo) ÷ (Nutrient intake) × 100. The data generated during the study was subjected to statistical analysis using SPSS Statistical Software (2011) Version 20.0 and results were interpreted.

RESULTS AND DISCUSSION

The average time expended by the horses to consume 1 kg concentrate mixture in different treatment groups i.e. T1 and T2 was observed to be 16±3 min and 17±5min, respectively. Horses remained healthy and the diets were eaten without any complications throughout the entire experimental period. High fat concentrate offered a good acceptability without any considerable difference in the palatability of diets in two groups. The effect of linseed oil incorporation on palatability of diets was supported by Delobel et al. (2008). Researches carried on effects of dietary linseed oil on palatability are quite limited in the literature. However, no negative consequence of feeding linseed oil, used as a source Omega-3 fatty acids,

on palatability of the diet was reported by Henry et al. (1991) and Hansen et al. (2002). Ribeiro et al. (2009) also did not observe any variation in acceptability by equines from a control diet and another with fat. While, few signs of mild colic, depression and anorexia were reported by Schumacher et al. (1997) at rates of linseed oil incorporation close to 2.5 mL/kg body weight (1250 ml/horse), that is, approx five times higher than the level used in the present trail. Hence, at reasonable inclusion rates, linseed oil could be assumed as safe to be supplemented in equine diets.

Horses consuming linseed oil diet were found to have significantly lower dry matter intake (Fig. 1) and organic matter intake (Fig. 2) than the horses consuming diet without inclusion of linseed oil. The results obtained are in corroboration with the observations of Bush et al. (2001), Meyers et al. (1989), Mattos et al. (2006) and Godoi et al. (2009). Marqueze et al. (2001) supplemented 7.8% soybean oil to horses and observed a decrease in dry matter intake (1.66% BW), similar to that reported by Kronfeld et al. (2004), found dry matter intake of 1.60% BW in several digestibility trials with hiperlypidemic diets. Mattos et al. (2006) observed reduction in dry matter intake in horses fed 3.1 and 6.8% soybean oil supplemented diet. Godoi et al. (2009) evaluated horses consuming diets without inclusion of soybean oil (control) and with addition of 8.5% and 19.5% soybean oil found that the dietary soybean oil addition increased significantly the diet energy density, with dry matter intake, expressed as a percentage of body weight, 1.80, 1.55 and 1.26% BW, respectively. It should be correlated with the fact that extra fat in the ration raises its energy density and that less dietary dry matter is required to satisfy the energy need of the horses which could lessen the chance of fermentation-related digestive disorders and reduce the weight load on performance horses.

Fig.1: Effect of linseed oil on dry matter intake (kg/d)

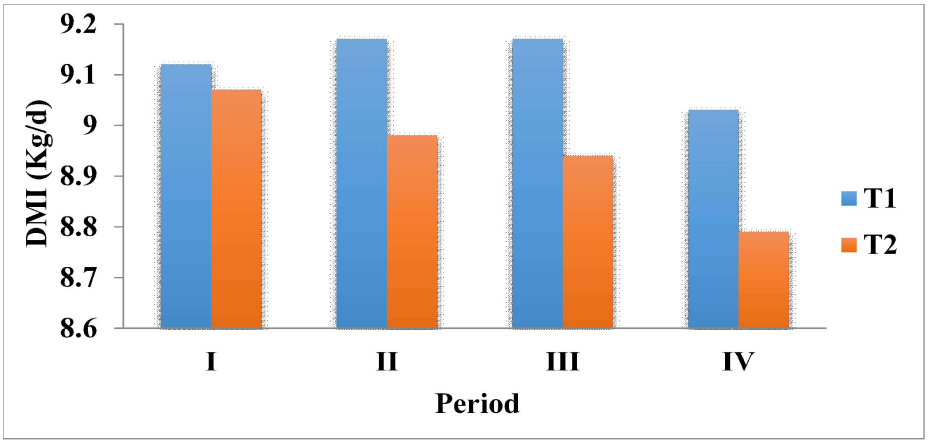
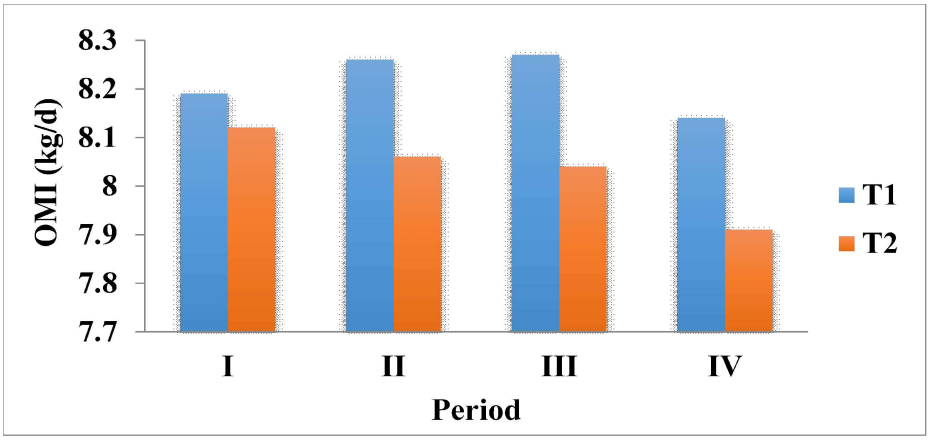


Fig 2: Effect of linseed oil on organic matter intake (kg/d)



The average initial body weight of Marwari horses distributed randomly in two treatment groups i.e. T1 and T2 were 377.50±20.65 and 368.75±15.34 kg, respectively. The mean fortnight body weights were found to increase from 377.50±20.65 to 389.50±14.79 kg and from 368.75±15.34 to 405.75±27.41 kg in T1 and T2 group, respectively (Fig.3). The statistical analysis of data revealed non-significant effect of incorporation of linseed oil on body weight in different treatment groups.

Fig 3. Effect of linseed oil on live body weight (kg)

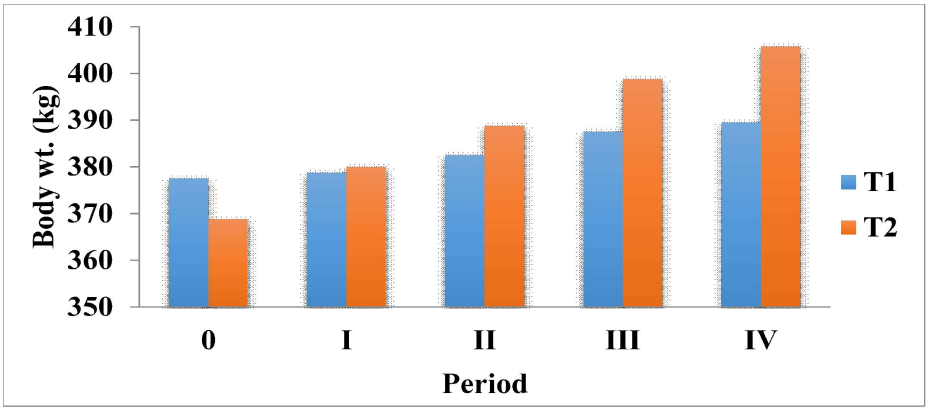


Table 3. Effect of linseed oil on digestibility coefficient of gross nutrients and fibre fractions in treatment groups

Attributes	Treatment Groups		Significance
	T1	T2	(P Value)
DM	60.3±0.22[a]	64.1±0.56[b]	0.001**
OM	66.9±0.66	68.7±0.78	0.115
CP	87.0±0.47	85.7±1.02	0.298
EE	62.4±1.82[a]	79.7±1.47[b]	0.000**
CF	39.5±2.01	42.8±0.88	0.180
NFE	61.1±0.76	63.1±0.25	0.071
NDF	48.8±0.74[a]	54.5±0.36[b]	0.002**
ADF	46.0±1.27	49.1±1.42	0.152

Note: Means with different superscripts in a row differ significantly from each other (P<0.01)

Digestibility of nutrients (Table 3) revealed significant (P<0.01) differences for DM, EE and NDF however, digestibilities of OM, CP and ADF were found to be non-significant. Higher dry matter digestibility of high fat diet when compared to control diet is in agreement with the results reported by Delobel et al. (2008), Mc Cann et al. (1987), Scott et al. (1989), Gobesso et al. (2009) and Potter et al. (2022). In other studies of Meyers et al. (1989), Lindberg and Palmgren Karlsson (2001) and Bush et al. (2001), no effects of the incorporation of fat on the digestibility of DM were found. In the present trial, the values of dry matter digestibility (60.31% and 64.13% for T1 and T2 treatment groups, respectively) were lower than those reported by Delobel et al. (2008) and this could be related to difference in the type of ingredients used in diet of horses.

Crude protein digestibility values were higher than Delobel et al. (2008) and Palmgren Karlsson et al. (2000) which may be attributed to differences in type and amount of feed ingredients used. The inclusion of linseed oil did not influence CP digestibility and is in agreement with Delobel et al. (2008), Bush et al. (2001), Lindberg and Palmgren Karlsson (2001) and Gobesso et al. (2009). Resende

et al. (2004) also supplemented 250, 500 and 750 mL of corn oil and there was no change in digestibility of crude protein. On the other hand, Meyer et al. (1997) and Jansen et al. (2002) found difference in the digestibility of CP with the addition of fat in the diet of horses and stated that the result could be related to the fact that diet intake with higher amount of fat can reduce the growth of bacteria in the large intestine, which would decrease the amount of fecal microbial protein, or the increased consumption of fat in the diet, which would reduce the apparent digestibility in the small intestine, due to increased flow of the endogenous nitrogen.

As expected, the inclusion of linseed oil in the concentrate improved the EE digestibility up to 79.72% as opposed to 62.55% for the control ration. Several authors reported an increase in apparent digestibility of EE when fat was added to the diet of horses and supported the present finding. Largest fat digestibility was observed when horses were fed 15% dietary fat (Bush et al., 2001). Delobel et al. (2008) concluded that the addition of linseed oil increased fat digestibility by 26.4%. The type of fat used for supplementation in the present study consisted of a vegetable oil, which is identified to be rich in triglycerides has been assumed to increase the proportion of easily digestible true fat (Delobel et al., 2008). Taking account of the suggestion by Kronfied et al. (2004) that enzymatic hydrolysis of fat is slow at low substrate concentrations in the small intestine, this could be assumed that increased proportion of substrate (dietary fat) leads to increase of lipolysis by enzymes. Moreover, an increasing amount of fat in diet was found to significantly increase the pre-ileal fat digestibility and jejuno-ileal flow of chyle in an experiment carried out by Meyer et al. (1997).

When going through the literature, there is controversy about the influence of high fat intakes on apparent crude fiber digestibility in horses. In the present study, the inclusion of linseed oil in the concentrate significantly increased the NDF digestibility in T2 group horses and these results are in agreement with the results reported by Delobel et al. (2008) who found a significant increase in NDF digestibility on inclusion of 8% linseed oil in the diet of horses. Likewise, Scott et al. (1987) found an increase in NDF digestibility when 5% and 10% fat was added in the diet of yearling horses and Potter et al. (2022) also observed a higher NDF digestibility in horses consuming fat-rich diet. Lindberg and Palmgren Karlsson (2001) found an increase in apparent digestibility of ADF and no effect of fat inclusion on total fibre digestibility. These differences in fibre digestibility could be due to the fact that the gastrointestinal system of horse follows an opposite digestive strategy compared to ruminants, with location of the large digestive fermentative chambers after the small intestine. The lipolysis for this supplemented fat should be entirely completed in the small intestine with no significant amount of fat entering the cecum to affect fiber digestibility (Delobel et al., 2008). Another point that should be considered is replacement of fermentable carbohydrates by fat would have decreased some suppressing effects of starch on fibre digestion (Palmgren Karlsson et al., 2000). Studies at Kentucky Equine Research (KER) have shown that pH of the hindgut drops significantly in horses following a starch rich diet as the feed which is high in starch content, will end up in accumulation of residual starch in caecum and colon where it is slowly fermented and may favour the growth of amylolytic bacteria and the growth of many fibre-fermenting microorganisms, such as *Ruminococcus albus* and *Fibrobacter succinogenes*, is suppressed. The conflicting results probably relate to the fact that the low-fat and high-fat diets used in the various studies (Table 4) differed with respect to multiple components, including the amount of crude fiber, forage/concentrate ratios or type/mode/level of fat incorporation. Furthermore, in a trial carried out by Williams et al. (2017), it was suggested that oil presented with concentrate could be less likely to coat fiber particles or could allow fat to be more digestible when compared to fiber only diet.

The values of DCP, TDN and ME were derived for experimental feeds in both treatment groups i.e. T1 and T2 from figures of chemical composition of experimental feed and average digestibility

coefficient of nutrients (Table 5). Value of DCP was higher in T1 as compared to T2 however, differences were non-significant. Whereas, TDN and ME values were significantly higher in T2 group as compared to T1. The recorded differences in digestibility of experimental feeds influenced their nutritive value as evidenced from DCP and TDN value. The ME value of the diet was calculated by applying the TDN (Kg) to ME (Mcal) conversion factor 3.608 as per NRC (2007).

Table 4. Effect of linseed oil on practical nutritional worth in different treatment groups (Nutritive value of diet/kg DM)

Attributes	Treatment Groups		Significance (P value)
	T1	T2	
DCP (g)	93.8±1.53	91.8±2.54	0.533
TDN (g)	626.3±2.96[a]	670.8±10.22[b]	0.006*
ME (Mcal)	2.26±0.01[a]	2.42±0.037[b]	0.006*

Note: Means with different superscripts in a row differ significantly from each other (P<0.01)

CONCLUSION

The data from the present research indicated that the inclusion of linseed oil in the diet of horses increased the energy density of diet while reducing the rate of feed intake. It could be concluded that, dietary supplementation of linseed oil at the rate of 8% in the concentrate mixture of horses is an easy and safe way to increase energy density of diet and at such inclusion rate, linseed oil improved the digestibility of DM, fat and NDF. Further researches should be conducted to analyse the effect of fat supplementation on equine gut microbiota and lipid interactions with other nutrients and minerals in the small and large intestine of horses with the goal of developing safer diets for horses.

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