The effect of dietary protein on reproduction in the mare. II. Growth of foals, body mass of mares and serum protein concentration of mares during the anovulatory, transitional and pregnant periods

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ABSTRACT

The effect of 4 different diets, in terms of protein quantity and quality, on total serum protein (TSP), albumin and globulin was investigated. Non-pregnant mares that were not lactating ($n = 36$), pregnant mares that had foaled ($n = 24$) and their foals ($n = 24$) were used in this study. Daily total protein intake had no effect on blood protein concentrations in the mares. Total protein intake and quality (available essential amino-acids) did affect the body mass of mares during lactation. When mares were fed the minimum recommended (National Research Council 1989) total daily protein, foal mass decreased by approximately 25 % at weaning compared to the foals whose dams were on a higher level of protein intake. The TSP concentrations of foals at birth were on average 10 g/ℓ lower than those of the mares. Albumin concentrations of foals during the first 60 days of life were on average 2–3 g/ℓ lower than those of the mares. Globulin concentrations of foals were approximately 5 g/ℓ lower than those of mares at weaning.

Key words: blood proteins, equine, foal growth, nutrition, protein.

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INTRODUCTION

The effect of nutrition on reproductive efficiency in ruminants is well known, but the nutrition of brood-mares has received very little attention in the past. Nutritional guidelines for brood-mares are limited and are in many cases based on standards published for ruminants without considering the inability of the horse to utilise low-quality protein in order to synthesise its own essential amino-acids[9]. Apart from lysine, it is unknown which of the amino-acids are essential for horses, but the amino-acids considered essential for pigs could be used as a guideline for horses owing to the similarity of the first parts of their digestive tracts (stomach and small intestine)[2]. Methionine supplementation has been shown to increase serum methionine, lysine, histidine, arginine, tryptophane, leucine and valine concentrations, and methionine can therefore be considered to be an essential amino-acid for the horse[2].

Another aspect that is usually overlooked is the necessity for specific essential amino-acids in the diet for the production of hypothalamic and hypophysial hormones[4]. A deficiency of these essential amino-acids could theoretically cause impaired synthesis of gonadotrophic hormones and indirectly also the synthesis of ovarian hormones, as well as growth hormone.

The inability of horses to utilise non-protein nitrogen (NPN) has been discussed[10]. In recent studies it was shown that foal growth was impaired with the inclusion of even low concentrations of NPN in the mares' ration, and mares also lost weight during the lactation period when NPN was included in the diet[6]. When the results of the first article in this series are considered, it is evident that the quality and quantity of protein in the diet of mares at stud should receive special attention[10].

The purpose of this report, the second of a series of 7, was to determine whether total protein intake and protein quality with respect to essential amino-acid content would affect total serum protein, albumin and globulin content in mares and growing foals. This study was conducted during the anovulatory, transitional and active breeding seasons as well as during pregnancy. Body-weight changes of both mares and foals were recorded and compared with their nutritional status.

MATERIALS AND METHODS

Thirty-six Thoroughbred and Anglo-Arab, non-pregnant, non-lactating mares that were covered and became pregnant and 24 pregnant mares that foaled and were subsequently covered were used in this series of trials. After the mares had foaled, the foals were included in this study until weaning at the age of 6 months.

This investigation was conducted on a farm in the Central Karoo region of South Africa. Minimum temperatures varied from –8 °C in winter to 1 °C in summer, while maximum temperatures varied from 1 °C in winter to 43 °C in summer.

The 36 non-pregnant, non-lactating mares were divided into 4 groups according to age and body weight and allocated to one of the 4 nutritional groups. The 24 pregnant, lactating mares were also divided into 4 nutritional groups according to age and expected foaling date. Four different diets were compiled as shown in Table 1. The experimental diets consisted of standard cubes, lucerne hay (*Medicago sativa*) or tef hay (*Eragrostis tef*) and fishmeal. The non-lactating mares received 2 kg of cubes per day throughout the current trial. After these mares had been covered they continued to receive the same diet until they foaled. The pregnant mares received their experimental diets from 6 weeks before their expected foaling dates, and were fed 2 kg of cubes/day, which was increased to 4 kg/day during the last month of pregnancy. The cubes were then increased to 7 kg/day 2–3 days after foaling and maintained at this level until the foals were weaned at the age of 6 months. Lactating mares received their cubes at 3 different times each day and, where applicable, their fishmeal in the morning feed. The non-lactating mares received 5 kg of either tef hay or lucerne hay as roughage

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daily throughout the experimental period, while the lactating mares received 7 kg of either tef hay or lucerne hay daily until their foals were weaned. The quantity of roughage was then reduced to 5 kg/day until the end of the experimental period. During the morning feed, foals were prevented from eating with the mares. When foals reached the age of 3 months they received 1 kg of cubes per day and approximately 2 kg of roughage, either tef or lucerne hay. After the foals were weaned at the age of 6 months, the mares were placed with the non-lactating mares in their respective groups. In the case of the 36 non-lactating mares the trial commenced in July 1989 and the mares were mated from 15 September until 31 December.

The groups were kept separately in 8 paddocks of 30 × 50 m. Individual feeding troughs were used for the concentrates while roughage was group-fed. During the period July 1989 – April 1990 the daily routine of the 36 non-lactating mares was as follows:

06:00 – Mares were teased and then tied individually to their feeding throughs.

07:00 – Blood samples were taken and mares were fed their concentrates, and fishmeal where applicable.

08:00 – Rectal and ultrasonographic examinations and matings were done.

11:00 – Mares received their roughage in hay racks.

The lactating mares were managed similarly except that their concentrates were fed in 3 equal portions at 08:00, 13:00 and 18:00 daily to reduce the risk of colic.

The non-lactating mares were weighed weekly until they were 30 days pregnant and on a monthly basis thereafter. Lactating mares and foals were weighed on the day of foaling and weekly thereafter until the foals were weaned at the age of 6 months.

All horses were regularly dewormed. The stallions and non-pregnant mares were inoculated with African horse sickness (AHS) Combination I and equine influenza vaccines in July 1989. In August 1989 all the stallions and non-pregnant mares were vaccinated with AHS Combination II and tetanus vaccines. In October 1989 both the stallions and all the mares were inoculated against equine influenza. After the mares had foaled they were vaccinated against AHS and tetanus. All the stallions were tested for fertility. All the experimental horses were tested for dourine before the commencement of this trial. Vaccines were supplied and dourine tests performed by the Onderstepoort Veterinary Institute, Onderstepoort, South Africa.

Table 1: **Daily diets** (kg) **of non-lactating** (NL) **and lactating** (L) **mares in 4 dietary groups.**

Feed component	Group							
	1		2		3		4	
	NL	L	NL	L	NL	L	N L	L
Cubes	2(4)[a]	6	2(4)	6	2(4)	6	2(4)	6
Lucerne hay			5	7			5	7
Tef hay	5	7			5	7		
Fishmeal					0.2	0.2	0.2	0.2

[a]Last month of pregnancy is indicated in brackets.

Table 2: **Mean daily crude protein intake** (g) **of non-lactating mares in 4 dietary groups.**

Period[a]	Group			
	1	2	3	4
1	806	1130	934	1258
2	714	1374	842	1502
3	676	1174	804	1302
4	706	1350	834	1478
5	782 (1066)[b]	1598 (1882)	910 (1194)	1726 (2010)
Mean	737	1325	865	1435

[a]Period 1 = July–September 1989: anovulatory; Period 2 = October–December 1989: breeding; Period 3 = January–March 1990: early pregnancy; Period 4 = April–June 1990: mid pregnancy; Period 5 = July–September 1990: late pregnancy.
[b]Value in brackets = increased protein intake during the last 3 months of pregnancy.

Analytical procedures

Samples of the 4 experimental diets were analysed for crude protein, total digestible nutrients (TDN) and the amino-acids threonine, methionine, iso-leucine, leucine, lysine and arginine using the methods described previously[10].

Total serum protein and albumin concentrations were determined spectrophotometrically according to the methods described[11]. The globulin content was calculated as the difference between TSP and albumin concentrations.

Blood sampling

Blood samples were obtained by venipuncture using 10 mℓ sterile evacuated blood collection tubes without anticoagulant (Vac-U-Test, Radem Medical). Blood samples were then centrifuged at 3000 rpm and the serum removed and stored in sterile 10 mℓ glass tubes at –20 °C.

Blood samples were collected from the experimental animals as follows:

Non-lactating mares: during the transitional period and breeding season from 1 July 1989 to 30 December 1989, 1 serum sample was collected from each of the 36 non-lactating mares every third week.

Lactating mares: after parturition, blood samples from the 24 mares and their foals were collected on the day of foaling and weekly thereafter for 60 days. From 60 days until weaning blood samples were collected every second week.

Statistical analysis

Group means and standard errors were calculated by one-way analysis of variance using the P7D programme of the BMDP statistical package[3] and the LSML 76 programme[5]. Protected least significant differences were calculated to compare treatment means.

RESULTS

The daily diets of the 36 non-lactating and 24 lactating mares are given in Table 1.

The mean daily crude protein intakes of the non-lactating and lactating mares are presented in Tables 2 and 3 respectively.

Period 1 (1 July – end September) is the anovulatory and transitional period, Period 2 (October – end December) the breeding season and Periods 3–5 the pregnant period.

In Table 3 the daily crude protein intake is given for the lactating mares during late pregnancy as well as from foaling until the foals were weaned. After weaning the mares received the same treatment (nutritionally) as the non-lactating mares.

In Table 4 the estimated average daily intake of 6 essential amino-acids in the 4 different diets are shown for the non-lactating and lactating mares.

In Fig. 1 the mean total serum protein (TSP), albumin and globulin concentrations of the non-lactating mares are shown for the period July to December. There were no differences recorded between the 4 nutritional groups in the mares and the foals and therefore only the mean results are given. In Fig. 2a–c the mean TSP, albumin and globulin concentrations are presented for the mares and foals during lactation. There was a distinct difference in TSP, albumin and globulin between mares and foals during this period. After parturition the TSP was approximately 10 g/ℓ lower in foals than the mares. This difference was maintained throughout the pregnant period. The foals had on average a 4 g/ℓ lower albumin concentration than the mares at parturition but this difference no longer existed after 70 d. Globulin concentrations were 6–7 g/ℓ lower in foals than in mares during the whole of the preweaning period. No differences in serum protein concentrations between groups were recorded during pregnancy.

The mean body mass of the non-lactating mares at the commencement of the trial was; Group 1, 427 ± 15; Group 2, 455 ± 14; Group 3, 434 ± 15, and Group 4, 434 ± 14 kg. The body mass of all the mares increased slightly during the period July to December. The mean increase in body mass for the different groups varied between 25 and 33 kg.

The lactating mares in Groups 2, 3 and 4 gained mass during lactation. This mean increase per mare during lactation was 28 kg (Group 2), 14 kg (Group 3) and 28 kg (Group 4). The mares in Group 1 lost on average 25 kg in body mass during lactation. The replacement of tef hay (Group 1) with lucerne hay (Group 2) and the supplementation of tef hay with 200 g of fishmeal (Group 3) prevented the loss of mass that was recorded in Group 1. Four mares in Group 1 lost weight and all 4 of these mares suffered embryonic loss before day 90 of pregnancy. Support will be provided for this statement in a follow-up publication.

The mass of the foals was similarly affected. The mean monthly mass of foals in the 4 groups is given in Table 5. During the first 10 weeks there was no difference in their mean mass in the 4 different nutritional groups. At weaning, the mean mass of foals in Group 1 was 153 kg in comparison to the mean mass of foals in Groups 2, 3 and 4, which was 200 kg, 182 kg and 202 kg respectively. The mean body mass of the lactating and non-lactating mares during pregnancy is given in Fig. 3. Lactating mares only started to gain weight after weaning.

Table 3: **Daily crude protein intake of lactating mares, during late pregnancy** (P – last 3 months) **and during the lactation period of 6 months** (L)**, in 4 dietary groups.**

	Daily crude protein intake (g)							
	Group							
Period[a]	1		2		3		4	
	P[b]	L[c]	P	L	P	L	P	L
1	1084		1384		1216		1516	
2	876	1214	1668	2138	1008	1278	1800	2202
3		1244		1937		1308		2001
4		1348		1950		1412		2014

[a]Period 1 = July–September 1989; Period 2 = October–December 1989; Period 3 = January–March 1990; Period 4 = April–June 1990.
[b]P = last 3 months of pregnancy.
[c]L = lactation period.

Table 4: **Mean daily amino-acid intake** (g) **of non-lactating** (NL) **and lactating** (L) **mares in 4 nutritional groups.**

	Amino-acid intake (g) of each group							
Amino-acid	1		2		3		4	
	NL[a]	L[b]	NL	L	NL	L	NL	L
Threonine	18.5	44.0	32.8	62.9	24.5	48.2	38.8	67.1
Methionine	6.9	16.5	6.1	15.8	11.5	19.7	10.5	19.0
Iso-leucine	24.6	55.4	42.7	78.5	31.5	60.3	48.7	83.4
Leucine	46.5	104.6	74.2	137.5	49.7	104.6	4.2	137.5
Lysine	36.0	74.8	51.0	95.8	47.9	83.9	62.5	104.9
Arginine	47.4	105.2	76.4	140.0	47.6	105.2	77.8	140.9

[a]NL = non-lactating (maintenance ration and first 6 months of pregnancy).
[b]L = lactating (6 months lactating period).

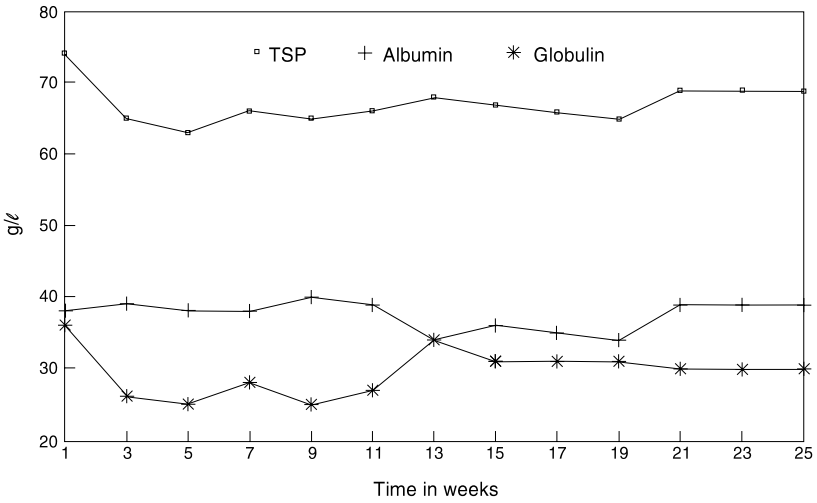

Fig. 1: **Mean total serum protein** (TSP) (g/ℓ)**, albumin** (g/ℓ) **and globulin** (g/ℓ) **concentrations of non-lactating mares** (NL) (*n* = 36) **in 4 nutritional groups for the period July to December.**

DISCUSSION

Protein intake

The crude protein intake of the non-lactating mares in Group 1 (Table 2) was the lowest of the 4 groups, but still in excess of 656 g/day that, according to the National Research Council (NRC), Washington DC[7] is the minimum requirement for a mare of 500 kg body mass. Comparing the daily total crude protein intake of the 4 nutritional groups during the period July to September it is evident that mares in Group 4 received nearly twice the

quantity of crude protein as the mares in Group 1. The replacement of tef hay in Group 1 by lucerne hay in Group 2 increased the daily protein intake to a greater degree than supplementation of the tef hay-based ration (Group 1) with 200 g fishmeal per day (Group 3).

The daily protein requirement of a mare of 500 kg during the first 3 months of lactation is estimated at 1427 g/day[7]. Lactating mares in Group 1 received 1214–1348 g of protein per day during this period. The average mass of these mares was 435 kg after parturition and according to the NRC standards, their daily diet met their protein requirements. Despite this, their average mass at weaning was approximately 410 kg.

Lysine is the only known essential amino-acid for horses[2]. According to the NRC[7] guidelines the lysine requirement for maintenance of a 500 kg mare is 23 g/day and increases to 28–30 g during the last 3 months of pregnancy. During the first 3 months of lactation the requirement increases to 50 g/day and thereafter declines to 37 g/day. Thus, according to these guidelines, mares in all 4 groups received sufficient lysine.

Definite differences in the essential amino-acid content of the 4 rations were found when the roughage components and the effect of fishmeal supplementation were taken into consideration. Replacement of tef hay (Group 1) with lucerne hay (Group 2) increased the intake of threonine, iso-leucine, leucine and arginine to a greater degree than supplementing tef hay (Group 1) with 200 g of fishmeal (Group 3) per day. In effect, both the lactating and the non-lactating mares in Group 3 (cubes, tef hay and fishmeal) had a lower intake of these amino-acids than the mares in Group 2 (lucerne hay and cubes) (Table 3).

The type of roughage, *i.e.* tef hay or lucerne hay had less effect on the intake of methionine and lysine than the other amino-acids. However, the supplementation of fishmeal (Diets 3 and 4) increased the total daily methionine intake by approximately 65 % in non-lactating mares and 20 % in the lactating mares (Table 3). A similar effect was achieved in the case of lysine (Table 3).

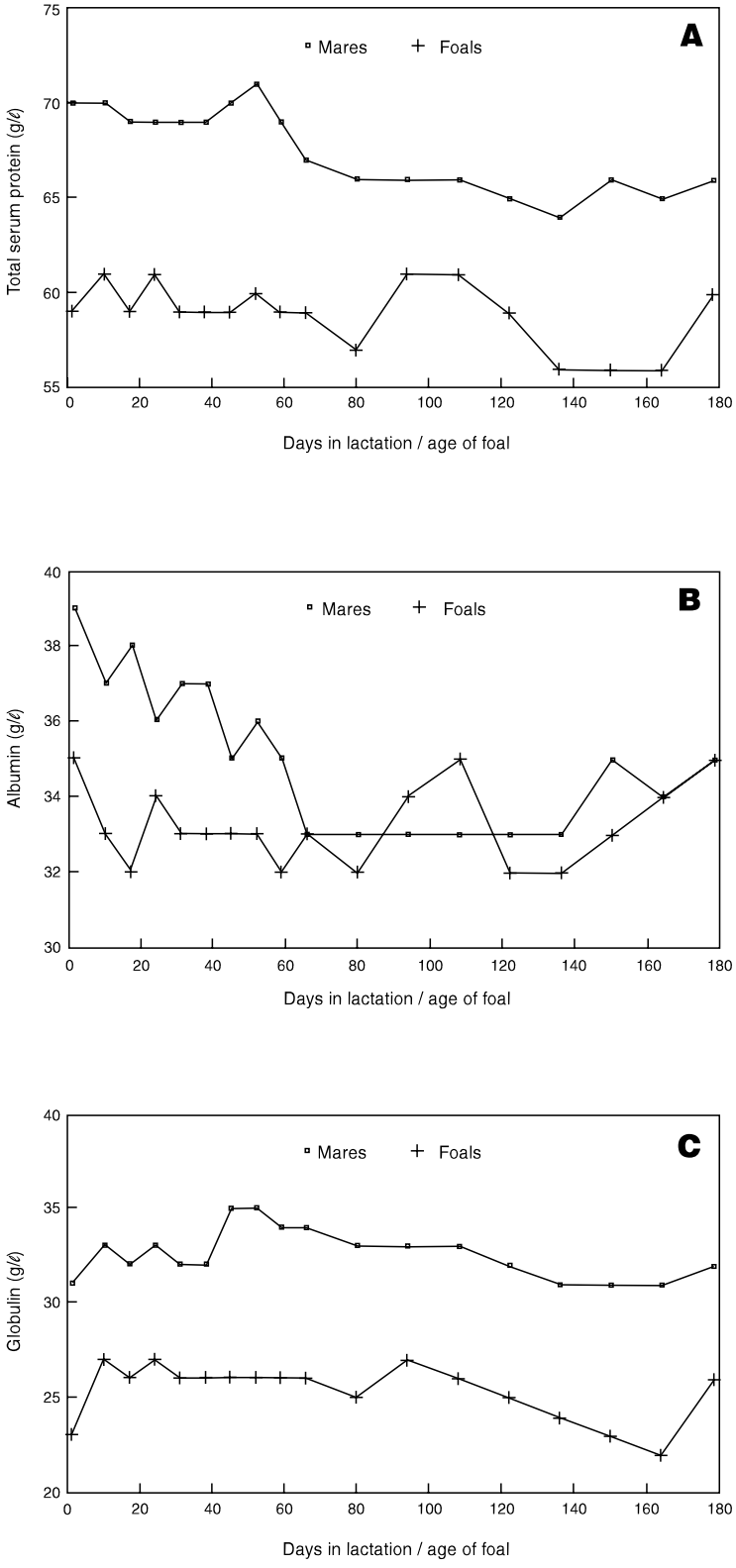

Fig. 2: **Mean total serum protein** (TSP) (g/ℓ) **(A), albumin** (g/ℓ) **(B) and globulin** (g/ℓ) **(C) concentrations of mares and foals in 4 nutritional groups during lactation.**

Body mass

Although the minimum protein requirements of the mares in Group 1 were met, the mares lost weight during early lactation. This resulted in early embryonic loss (4 of 6 mares) (reported later in this series of papers) and the growth of their foals was impaired. Foals of mares in Group 1 weighed up to 25 % less (153 kg versus 182–202 kg) than the foals in the other 3 groups, which may indicate that foals weaned with a low body mass are more likely to have periods of rapid compensatory growth at a later stage and may consequently be highly susceptible to epiphysitis.

As the main objective of this study was to investigate the effect of protein nutrition on reproduction, the energy content was kept as constant as possible in the 4 rations. This was achieved by the low variation of 4 % in the TDN values of 62–66 %.

Table 5: **Mean body mass** (kg ± SD) **of the foals of mares in 4 dietary groups from birth** (week 0) **until weaning** (week 25)**.**

	Group			
Age in weeks	**1**	**2**	**3**	**4**
1	47.3 ± 7.45	47.2 ± 4.97	51.25 ± 10.99	46.67 ± 3.67
5	73.83 ± 9.78	76.4 ± 7.89	83.25 ± 8.38	76.17 ± 10.30
10	96.33 ± 10.	107.2 ± 3.63	109.5 ± 17.06	108.33 ± 7.00
15	110.67 ± 13.99	143.44 ± 8.32	138.75 ± 16.68	138.0 ± 9.59
20	136.4 ± 14.50	170.0 ± 11.55	164.33 ± 25.42	173.0 ± 9.02
25	153.0 ± 16.91	200.4 ± 10.52	182.33 ± 28.11	202.2 ± 10.35

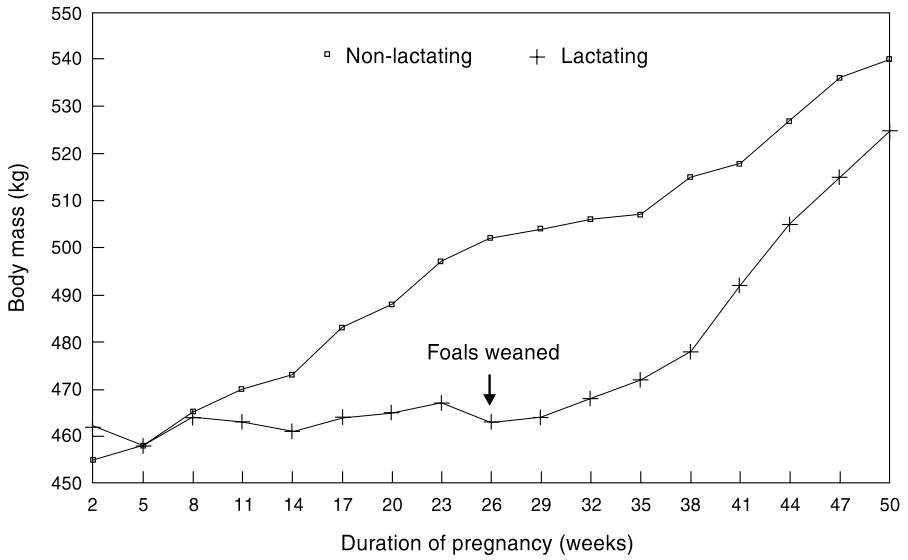

Fig. 3: **Mean body mass** (kg) **of non-lactating** (NL) **and lactating mares** (L) **in 4 nutritional groups during pregnancy.**

Total serum protein (TSP) concentrations

The normal TSP concentration for horses is given as 60–77 g/ℓ, albumin between 29–38 g/ℓ and globulin 25–53 g/ℓ[1]. Results obtained in this study with non-lactating mares were within these normal values. No significant differences were observed between the nutritional groups and therefore only the mean values of the 4 groups are presented (Fig. 1). It is evident that serum protein concentrations were not directly influenced by dietary protein intake even at the levels fed to Group 1 in this experiment. Doubling the protein intake (Group 1 vs Group 4) also had no effect on serum protein concentrations, which confirms that serum proteins are produced mainly by the liver, and as long as the minimum protein intake is maintained, the serum protein concentrations should remain constant, provided that internal parasites are not a problem[8].

No differences in the TSP, albumin and globulin concentrations were observed between nutritional groups in the lactating mares or the foals respectively during lactation. Therefore the results of the different nutritional groups were pooled and the mean values are presented in Fig. 2a–c. Shortly after birth the average TSP concentrations of the foals were approximately 10 g/ℓ lower than those of the mares. These concentrations increased to values that were only 5 g/ℓ lower than the mares at weaning, primarily due to the increase in albumin concentrations. The albumin concentrations of the foals were approximately 4 g/ℓ lower than those of the mares at parturition but increased to the same concentrations as the mares within 70 days after birth. The globulin concentrations of the foals, however, were 6–7 g/ℓ lower than those of the mares throughout lactation, which could be explained by a lower level of immunity in the foals.

The TSP concentrations of lactating and non-lactating mares had a similar pattern. In addition, the total protein intake had no effect on serum protein concentrations and the concentrations of the 4 different nutritional groups varied within the normal range of 60–70 g/ℓ.

CONCLUSION

These results show clearly that the total protein intake does not affect the total serum protein, albumin or globulin concentrations provided that the minimum protein requirements are met. In brood-mares and growing foals on a maintenance feeding programme, the serum protein concentration is not affected by the total intake of essential amino-acids. However, foal growth during lactation is influenced significantly by the quality of protein ingested by the mare. The dietary protein intake of the lactating brood-mare should therefore contain sufficient high-quality protein to prevent depletion of her body reserves and consequently a loss in body mass with resultant early embryonic loss.

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