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Original article

Blood serum and skin fatty acid levels in horses and the use of dietary polyunsaturated fatty acids[1]

Domenico BERGERO[a]*, Nicoletta MIRAGLIA[b], Mimmo POLIDORI[b], Marisa ZIINO[c], Daniele GAGLIARDI[d]

[a]Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy
[b]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, University of Molise, Via De Sanctis, 86100 Campobasso, Italy
[c]Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy
[d]Veterinary Practitioner, Campobasso, Italy

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Abstract — The effect of the addition of a daily 20 g dose of an unsaturated fatty acid rich oil from Purple Viper's Bugloss seeds (Crossential SA 14, Croda) has been studied on saddle horses fed a near to maintenance level of a first cut meadow hay – barley diet. The percentages of the following fatty acids: C16:0, C18:0, C18:1, C18:2 ω6, C18:3 ω3, C18:4 ω3, C20:3 ω6, C20:4 ω3, C20:4 ω6, C22:5 ω3, C22:6 ω3, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, ω3 fatty acids, ω6 fatty acids, were determined in blood serum and skin. The ω3/ω6 ratio was also considered. No statistical differences were found for the treatment. In the skin, compared with blood serum, a higher percentage of C16:0, C18:1, C18:4 ω3, monounsaturated fatty acids, ω3 fatty acids and saturated fatty acids were found; the ω3/ω6 ratio was also higher in the skin. Lower percentages of C18:0, C18:2 ω6, polyunsaturated fatty acids and ω6 fatty acids were also recorded for the skin, compared with blood serum. The blood levels of fatty acids were not always similar to the values found in the literature, but some differences can be explained by the differences in the diets used. The low level of oil addition can explain the lack of significance of the effect of the oil used. Further studies are needed to confirm the trend in the differences found between blood serum and skin percentages of fatty acids.

horse / fatty acids / Purple Viper's Bugloss / blood serum / skin

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*Correspondence and reprints
Tel.: 39 11 6709207; fax: 39 11 6709240; e-mail bergero@veter.unito.it

Résumé — Concentrations sérique et cutanée des acides gras lors de l'addition d'acide gras polyinsaturés dans la ration alimentaire des chevaux. Nous avons étudié l'effet de l'addition d'une dose de 20 g d'une huile riche en acides gras insaturés extraits de l'herbe à vipère (*Echium plantagineum*) (Crossential SA 14, Croda) dans la ration de chevaux de selle alimentés avec un foin de pré et de l'orge floconné ; le niveau alimentaire était proche de la ration d'entretien. Dans le sérum et dans la peau, ont été déterminés les pourcentages des acides gras suivants : C 16:0, C 18:0, C 18:1, C 18:2 ω6, C 18:3 ω3, C 18:4 ω3, C 20:3 ω6, C 20:4 ω3, C 20:4 ω6, C 22:5 ω3, C 22:6 ω3 ; ainsi que les pourcentages totaux des acides gras saturés, monoinsaturés, et polyinsaturés, des acides gras ω3, des acides gras ω6 et du rapport ω3/ω6. Les résultats obtenus en comparant les deux traitements ne sont pas significativement différents. Les concentrations des acides gras de la peau, comparativement à celles du sérum, ont eu des valeurs plus élevées pour les AG suivants : C 16:0, C 18:1, C 18:4 ω3, pour les acides gras monoinsaturés, pour les acides gras ω3 et pour les acides gras saturés. De même, le rapport ω3/ω6 est significativement plus élevé dans la peau. En revanche, nous avons observé des valeurs plus faibles dans la peau pour les AG : C 18:0, C 18:2 ω6, pour la somme des acides gras polyinsaturés et pour les acides gras ω6. Les valeurs des acides gras sériques observées sont différentes par rapport aux données de la littérature, mais cette différence peut être expliquée par des différences entre les régimes alimentaires administrés. L'absence d'effet significatif par rapport à l'effet de l'addition d'huile peut être due à la faible quantité d'huile administrée.

cheval / acides gras / herbe à vipère / sérum / peau

1. INTRODUCTION

When normal feeding of a hay based diet is offered to stabled horses this type of feed does not provide the animals with many unsaturated fatty acids, but it can if it is supplemented with concentrates in relationship to type and quantity [17]. The feeding affects the plasma levels of fatty acids whereas various researchers have noted great differences in plasma levels under normal conditions. For this reason, it is important to assess the fatty acid composition of the diets given to horses when studying their plasma fatty acid composition. The relative percentage of adipose tissues in the carcass of horses, reaches 9% at 30 months of age [10] and the lipid content of meat ranges from 0.5 to 3.0% [14]. The fatty acid composition of the horse's adipose tissue has been extensively studied [2, 12–15]. Namely, Pitre [14] reports the following percentages of saturated and unsaturated fatty acids in this tissue: C10:0, 0.05%; C12:0, 0,15%; C14:0, 4.2%; C15:0, 0.3%; C16:0, 27.9%; C16:1, 6.1%; C17:0, 0.4%; C18:0, 6.0%; C18:1, 37.0%; C18:2, 7.3%; C18:3, 9.0%; C20:0, 0.5%.

There is a disagreement about the optimum levels of plasma fatty acids to be reached in horses and their subsequent effects [7, 8, 11], but it is stated that fatty acids, particularly polyunsaturated acids, can play an important role in energy metabolism, in the modulation of the inflammatory process in general [20], joint inflammation [16], in endotoxaemia [4], respiratory allergies [19], maintaining a healthy skin and also in skin inflammation [1, 6]. With respect to this, it is important to assess the effect of different levels of fatty acids, especially polyunsaturated, in the various parts of the horse's body when added to the horse's standard feed. The exercise also affects the fatty acid blood serum levels, as confirmed by Vermorel et al. [18].

This first paper is primarily concerned with the addition of a limited amount of an oil rich in unsaturated fatty acids (Crossential SA 14, Croda) and the effects this addition can have on the relative blood serum and skin concentrations in saddle horses.

The interest of the skin fatty acid profile is connected with the known effect that polyunsaturated fatty acids have on the skin

of horses and the prevention of skin ailments and disease: the products used have a profile which we expect could have beneficial effects on the skin of horses.

2. MATERIALS AND METHODS

2.1. Animals

The experiment was conducted on four riding horses (two adult geldings, one two-year old filly and one mare of the "Italian saddle" breed), 426 ± 35 kg body weight (BW). The horses received "light exercise" daily according to the INRA scale [9]. Averaged total requirements reached 6.4 ± 0.3 UFC.

2.2. Feeds and feeding

The horses were fed daily (at 9.30, 12.30 and 18.30) 5.6 ± 0.7 kg dry matter of first cut meadow hay (7.2% crude protein, 0.5 UFC·kg^{-1}, 34% crude fibre on a dry matter basis) and 3.1 ± 0.4 kg dry matter of barley (10.0% crude protein, 1.16 UFC·kg^{-1}, 5.4% crude fibre on a dry matter basis). The nutritive level was 1.68 ± 0.01, referring to maintenance requirements [9]; the daily dry matter intake was 8.7 ± 1.1 kg; the total net energy intake was 6.4 ± 0.3 UFC. The energy requirements were met, by modulating the feed intake, at every stage of the trial.

Crossential SA 14 (Croda) is Purple Vipe's Bugloss (*Echium plantagineum*) oil. Purple Viper's Bugloss is a high purity plant seed lipid containing essential fatty acids (EFAs) and stearidonic acid (SA). Lipids extracted from *Echium plantagineum* seeds are rich in SA (approximately 14%) and the content of EFAs is around 47%. Its skin safety profile is expected to be similar to Borage (*Borago officinalis*) and Blackcurrant (*Ribes nigra*) oils, which are already used in skin care applications [3]. The composition in fatty acids of Crossential SA 14 (Croda) is designed in Table I.

2.3. Experimental design and measurements

Two of the four horses, during the first 30 days of the experiment, were given 20 g·day^{-1} (0.047 g·kg^{-1} bw) of Crossential

Table I. Relative fatty acid composition (as percent) of the Vipe's Bugloss (*Echium plantagineum*) oil used in this trial (Crossential SA 14).

Fatty acid	Relative percentage
C 16:0 (palmitic acid)	7.3
C 16:1 (palmitoleic acid)	0.1
C 16:2 (palmitolenic acid)	0.1
C16:3 (palmitoleidic acid)	0.1
C18:0 (stearic acid)	4.3
C 18:1 ω9 (oleic acid)	16.1
C 18:1 ω7 (vaccenic acid)	0.6
C 18:2 ω6 (linoleic acid)	18.2
C 18:3 ω6 (γ linolenic acid)	10.4
C 18:3 ω3 (α linolenic acid)	28.7
C 18:4 (stearidonic acid)	12.4
C 20:0 (arachidic acid)	0.2
C 20:1 (gadoleic acid)	0.7
C 22:0 (behenic acid)	0.1
C 22:1 (erucic acid)	0.2
C 24:1 (nervonic acid)	0.1

SA 14 (Croda) mixed with the concentrate. Then, the other two horses were given the same amount of Crossential SA 14 (Croda), whereas the first two horses were offered a normal diet during the next 30 days of the experiment.

After 30 and then 60 days of oral administration, blood (from the jugular vein) and skin samples were collected at 9 am from each horse, to avoid interferences due to the circadian differences in fatty acid blood levels [11] and meal supply. The blood samples were centrifuged. The percentages of different fatty acids were determined in all serum and skin samples.

Chemical composition of feedstuffs was analysed using a forced ventilation oven, a Kjeldahl apparatus for crude protein and a Fibertec apparatus for crude fiber.

The lipid fraction of the serum was extracted after it was freeze-dried with a mixture of chloroform and methanol, according to the Folk method modified by Ways and Hanahan [5]. The skin lipid fraction was obtained by continuous current extraction with a Soxhlet apparatus.

The lipids were extracted using petroleum ether for about 8 hours. Lipids of the different samples were then converted to methyl esters, which were then analyzed with a gas chromatography method, using a DANI. 8610 Gas Chromatograph equipped with a hydrogen flame ionization detector, and a 30 m × 0.32 mm i.d. fused silica capillary Omegawax 320 column (Supelco) having a 0.25 μm film thickness.

2.4. Statistical analyses

Data concerning the percentages of single fatty acids, particularly the ones concerning: C16:0, C18:0, C18:1; C18:2 ω6; C18:3 ω3, C18:4 ω3, C20:3 ω6; C20:4 ω3; C20:4 ω6; C22:5 ω3; C22:6 ω3; saturated fatty acids; monounsaturated fatty acids; polyunsaturated fatty acids; ω3 fatty acids; ω6 fatty acids; ω3/ω6 ratio, were subjected to a two ways multifactorial variance analysis, considering the treatment and material as factors.

3. RESULTS AND DISCUSSION

Table II shows means and standard deviations of the data resulting in the single cells. In particular, in this table the normal values of the horse not treated with the oil, are presented in the "control" column. Individual variability is shown by standard deviation.

The percentage of fatty acids found in blood serum in our study are only partly in accordance with what is mentioned in the bibliography. In our study, palmitic and stearic acid percentages were higher whereas the percentage of α-linolenic acid was lower even with the addition of the oil. In particular, Gazzola et al. [7] reported values of C18:1 ranging from 15 to 42%, those of C18:2 ranging from 28 to 37% and those of C18:3 ranging from 0 to 3% in the blood serum of horses fed diets with between 3 and 20% crude fat of a non-specified origin. Namely C18:1 percentage is far higher than the percentage we observed: 11.3%, whereas our values of C18:2 and C18:3 were found to be within the range of those reported by the Gazzola et al. [7] study. There were substantial differences in the saturated fatty acids in our study due to the higher levels of palmitic and stearic acids. It is likely that the differences were due to varying intakes of fatty acids in the horse's diet.

Gene Luther et al. [8], reported a composition of blood plasma of horses fed with a pellet diet with doses of 12.5 $g \cdot kg^{-1}$ $PV \cdot day^{-1}$. In this study the percentage of C18:1 of 14.7% was again higher than our values, whereas C18:2 was 44%, which is 10 points higher than our data. Orme et al. [11], feeding 2 kg of horse cubes containing 4–4.25% oil per horse per day and 4–6 kg hay per horse per day, measured 2.5%

Table II. Percentages of fatty acids in the blood serum and skin treated with Vipe's Bugloss (*Echium plantagineum*) oil administration (Treatment group) or not (control group); means (standard deviation.

Fatty acid	Blood serum, control	Blood serum, treatment	Skin, control	Skin, treatment
C 16:0	19.84 ± 2.67	20.13 ± 2.69	35.77 ± 1.82	32.80 ± 3.04
C 18:0	21.89 ± 2.56	20.92 ± 1.95	15.50 ± 1.36	15.97 ± 1.40
C 18:1	11.0 ± 1.64	11.35 ± 1.31	13.29 ± 1.34	13.22 ± 1.43
C 18:2 ω6	33.97 ± 3.04	34.95 ± 2.82	7.14 ± 2.67	6.58 ± 2.63
C 18:3 ω3	1.03 ± 0.36	1.11 ± 0.35	0.23 ± 0.45	0.71 ± 1.13
C 18:4 ω3	0.20 ± 0.19	0.18 ± 0.09	2.76 ± 1.01	3.06 ± 1.19
C 20:3 ω6	0.74 ± 0.23	0.90 ± 0.43	0.82 ± 0.59	0.93 ± 0.40
C 20:4 ω3	0.25 ± 0.21	0.14 ± 0.11	0.36 ± 0.54	0.56 ± 0.49
C 20:4 ω6	1.27 ± 0.41	1.64 ± 0.16	0.84 ± 0.64	1.64 ± 1.16
C 22:5 ω3	0.24 ± 0.05	0.27 ± 0.14	0.06 ± 0.12	0.89 ± 1.55
C 22:6 ω3	0.94 ± 0.60	0.65 ± 0.42	1.50 ± 1.12	0.58 ± 0.52
Saturated	44.86 ± 2.33	43.46 ± 1.56	64.90 ± 2.48	61.34 ± 3.09
Monounsaturated	13.07 ± 1.74	10.35 ± 6.46	18.08 ± 1.78	18.38 ± 1.75
Polyunsaturated	38.93 ± 3.23	40.12 ± 2.45	14.79 ± 2.42	16.19 ± 1.80
Poly. ω3	1.72 ± 0.68	1.70 ± 0.50	3.41 ± 0.72	5.22 ± 2.11
Poly. ω6	36.19 ± 3.05	37.73 ± 2.63	9.88 ± 2.89	10.38 ± 3.52
ω3/ω6 ratio	0.07 ± 0.03	0.06 ± 0.01	0.56 ± 0.31	0.67 ± 0.45

linolenic acid, 9.3% linoleic acid, 8.6% oleic acid at 9 am: the only value in accordance with that of our study is that of oleic acid; Orme et al. [11] found higher α-linolenic acid and lower linoleic acid concentrations.

In our study, we found lower percentages of C18:1 and C18:3, higher values for C18:0 and C18:2 in blood serum, and comparable values for C16:0 and C18:2 in the skin as compared with the composition of meat, as reported by Pitre [14].

Statistically significant differences were found between the blood serum and skin regarding the percentages of C16:0, C18:0, C18:1, C18:2 ω6, C18:4 ω3, monounsaturated fatty acids, polyunsaturated fatty acids, ω3 fatty acids, ω6 fatty acids, saturated fatty acids and ω3/ω6 ratios (Tab. III).

As shown, skin had a higher concentration than blood serum, namely for C16:0 (control: +15.93%; treatment: +12.67%), C18:1 (control: +1.99%; treatment: +1.87%), C18:4 ω3 (control: +2.56%; treatment: +2.88%), monounsaturated fatty acids (control: +5.01%; treatment: +8.03%); ω3 fatty acids (control: +1.69%; treatment: +3.52%) and saturated fatty acids (control: +20.04%; treatment: +17.88%). The ω3/ω6 ratio was also higher (control: +0.49%; treatment: +0.61%).

The percentages of C18:0 (control: –6.39%; treatment: –4.95%), C18:2 ω6 (control: –26.83%; treatment: –28.37%), polyunsaturated fatty acids (control: –24.14%; treatment: –23.93%) and ω6 fatty acids (control: –0.43%; treatment: –0.00%) were indeed lower in the skin than in blood.

Undoubtedly, there is selected inclusion of fatty acids in the skin with a significant preference with regards to ω3 fatty acids compared to ω6. It is evident that there is a dramatic reduction of linoleic acid with an increase in monounsaturated fatty acids.

The analysis of variance for the considered percentages and ratio did not show any

Table III. Statistical significance between blood serum and skin.

Fatty acid	Significance
C16:0	($P < 0.001$)
C18:0	($P < 0.001$)
C18:1	($P = 0.020$)
C18:2 ω6	($P < 0.001$)
C18:4 ω3	($P < 0.001$)
monounsaturated fatty acids	($P = 0.003$)
polyunsaturated fatty acids	($P < 0.001$)
ω3 fatty acids	($P = 0.001$)
ω6 fatty acids	($P < 0.001$)
saturated fatty acids	($P < 0.001$)
ω3/ω6 ratio	($P = 0.002$)

significant difference due to the treatment. Only saturated fatty acid percentages were low and close to the lower significance ($P = 0.064$), both in blood serum and in the skin after oil administration. No significant difference was found in the interaction between the two considered factors, for either of the studied parameters.

The treatment then, at the studied doses, that were near the levels commonly used, did not influence the percentages of fatty acids in the two biological matrices studied: blood serum and skin. Table II shows the values for the single fatty acids and individual variability. A discussion about the metabolic pathway of the absorbed fatty acids, that in this first step was not considered, could be very interesting.

These data considered on the whole confirm the importance of a nutritional influence in determining the percentages of blood fatty acids. In our study the lack of an effect can be explained by the low dose levels administered. The daily intake obtained by the addition of oil was 3.22 g of oleic acid, 3.64 g of linoleic acid, and 5.74 g of α-linolenic acid, and lower doses of other fatty acids. In these conditions, even the base diet intake, above all linoleic acid (over 60 $g \cdot day^{-1}$ from data tables) and α-linolenic acid (over 20 $g \cdot day^{-1}$ from data tables), can be considered significant. Some other parameters seem to be more important in the setting of blood serum fatty acids: in particular, training can affect this parameter [18].

Further ongoing studies will allow to verify the results obtained and indicate the effect of the addition of higher doses of fatty acids. For the verification of metabolic pathways, particularly those of polyunsaturated fatty acids, we suggest studying the administration of mixed ω3 and ω6 parent compounds alone (linoleic and α-linolenic acids).

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