

PROOF OF CONCEPT STUDY PROTOCOL

For: Keragenix Biotech AG

Study No.: KGB E 25032 A Herd Study and
KGB E 25032 A 25032 A Case Study

Study Title:	A Proof of Concept Study to Evaluate Hoof Growth, Moisture Content, and Structural Integrity in Horses Treated with Topical Sodium Cromoglycate-based Hoof Cream.
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Any other personnel involved with the study will be identified in the raw data. The Investigator will assure that study personnel are by training and experience, capable of performing the procedures called for in this Protocol.

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1. OBJECTIVE

This study aims to assess the outcomes listed below following treatment with a patent-protected hoof cream featuring sodium cromoglycate:

- Quantify hoof wall growth over 35 days
- Measure moisture
- content change using practical field tools
- Assess hoof structural quality and general condition
- Observe therapeutic effects in a horse with severe hoof issues over an extended period

2. JUSTIFICATION

Horses suffer from a number of hoof disorders and diseases. A 2017 prevalence study of hoof disorders seen in horses at hoof trimming in the Netherlands found that 85% of horses were affected by at least one hoof disorder¹. Furthermore, a 2017 review study in the United Kingdom found that 89% of respondents had encountered hoof abnormalities in their horse². Hoof abnormalities, disorders and diseases include superficial hoof wall cracks, perforating hoof wall cracks, horizontal hoof cracks, sole bruises, growth rings, limited growth, laminitis, thrush and white line disease¹. Poor circulation and inflammation results in slow hoof recovery. All of these issues lead to reduced performance of the horse, increased veterinary costs and decreased quality of life.

The Sodium Cromoglycate-based Hoof Cream has demonstrated positive results in improving hoof health, including growth rate, moisture retention, and resistance to cracking. This equine-focused study will measure these effects in horses over a 35-day period using practical, validated methods. A supplemental clinical case arm will evaluate the cream's use in horses with severe hoof issues over a 60-90 day period.

3. COMPLIANCE

The study will comply with the principles of good scientific practice and relevant Invetus SOP's.

4. TEST SITE

Animal Phase:

Save a Horse Australia (SAHA)
51 Wesley Way
Gleneagle
QLD 4285

5. STUDY DATES

The study start date will be subject to Protocol approval, animal welfare approval, availability of suitable study animals, and availability of the Investigational Veterinary Product (IVP). Actual start and finish dates will be included in the Study Report.

Estimated start date (animal phase): AUG 2025

Estimated finish date (animal phase): DEC 2025

6. STUDY DESIGN

6.1 Design

The overall design of the study will be a randomized, controlled study with an observational case study extension. The assessing veterinarian and farrier will be blinded, while Invetus personnel, monitor and SAHA staff will be unblinded.

6.2 Experimental Unit

The experimental unit will be the individual horse.

6.3 Experimental Models

The primary cohort in the herd study will be 20 horses, and the case study arm will include 1-5 horses. All horses will be between 2 and 20 years of age, and of any breed and weight, however, horses in the case study arm will have severe hoof pathology (including but not limited to wall cracks, minimal growth, poor hoof quality).

6.4 Inclusion, Exclusion and Removal Criteria

Animals will be selected for the study if they meet the criteria outlined below. Animals that are debilitated, suffering from disease or injury (excluding severe hoof pathology), fractious or otherwise unsuitable for inclusion in the study, in the opinion of the Investigator, will be excluded. Horses that become unhealthy during the conduct of the study or become unsuitable for sampling can be excluded by the Investigator in association with the veterinarian and in consultation with the Sponsor Representative. Reasons for exclusion will be documented.

Primary/main cohort in the herd study:

Inclusion:

- 2-20 years of age
- Clinically healthy with no systemic illness
- Balanced hooves at Day 0 (trimmed)
- Have had no hoof products in the 5 days prior to Day 0
- Horses have a temperament that is amenable to veterinary/farrier examination and study treatment

Exclusion:

- Hooves with active infections
- Hoof capsule distortions due to chronic lameness
- Current systemic medication impacting hoof health

Case Study:

Inclusion:

- 2-20 years of age
- Clinically healthy with no systemic illness
- Severe hoof pathology (including but not limited to wall cracks, minimal growth, poor hoof quality)
- Horses have a temperament that is amenable to veterinary/farrier examination and study treatment

Exclusion:

- Hooves with active infections
- Current systemic medication impacting hoof health

6.5 Allocation

The method of allocation and randomisation will be described in the raw data and the Study Report.

7. INVESTIGATIONAL VETERINARY & CONTROL PRODUCTS

All IVP details including batch number, expiry date, receipt and usage will be recorded on the “Drug Reconciliation” form.

7.1 Investigational Veterinary Product (IVP)

Name: Keragenix Biotech Hoof Cream

Batch No.: To be documented in raw data

Composition: Active ingredient: Sodium Cromoglycate.
Supporting ingredients:
Centella Asiatica, Olive Oil, Shea Butter,
Beeswax, Tea Tree Oil, Borax

Expiry Date: To be documented in raw data

Dose Level: Small amount to cover the coronary band

7.2 Control Product (CP)

Not applicable.

7.3 Source

The IVP will be sourced and supplied by the Sponsor.

7.4 Storage

The storage location and conditions will be recorded on the “Drug Reconciliation” form. The IVP should be stored at ambient temperature.

7.5 Safety

A Safety Data Sheet for the IVP will be sources and included in the study data.

7.6 Drug Disposal

The disposal of all remaining IVP will be documented according to SOP STU-308.

8. TREATMENT

8.1 Dose Calculation, Preparation & Administration

Study animals will be dosed according to the treatment regime detailed in Table 1A or 1B below.

Table 1A: Treatment Regime for the Primary Cohort in the Main Herd Study

Group	IVP Details	Dose Volume (per hoof)	Route	Freq.	Trt. Day(s)	No. Anim.
1 - Control	Untreated	N/A	N/A	N/A	N/A	10
2- Treatment	Keragenix Biotech Hoof Cream	Approximately tablespoon	Topical to the coronary band	Once daily ¹	35 days +/- 3 days	10

Table 1B: Treatment Regime for the Case Study

Grp.	IVP Details	Dose Volume (per hoof)	Route	Freq.	Trt. Day(s)	No. Anim.
3- Treatment	Keragenix Biotech Hoof Cream	Approximately tablespoon	Topical to the coronary band	Twice daily (AM and PM) ²	Minimum 60 days ³	1-5

¹ Morning treatment will not be applied on Days 0, 7, 14, 21, 28 or 35

² Morning treatment will not be applied on Days 0, 35, 56 or 84

³ With the possibility of being extended to 90 days

Treatment administration will be recorded on the “Animal Treatment Record”. The hoof will be cleaned of excess mud and dirt. Once cleaned, using a gloved hand, apply approximately a tablespoon of the IVP to the coronary band. For horses in the Primary Cohort in the Main Herd Study, all four hooves will be treated,

however, only the near front hoof will be assessed. For horse(s) in the Case Study Arm, all four hooves will be treated and assessed.

9. SCHEDULE OF EVENTS

Table 2: Schedule of Events for the Primary Cohort in the Main Herd Study:

Approx. Study Day	Event
Pre-study	Recruit site and cooperators Finalise study plan Receipt of IVP. Receipt of Animal Ethics Committee approval.
Pre-treatment	Screen horses for inclusion criteria Train co-operators
Approx. Day -5	Veterinary examination
On day -5 or before day 0	Randomise to treatment groups
Day 0	Veterinarian assesses hoof moisture and undertakes visual hoof assessments Farrier trims hooves, mark coronary band and undertakes visual hoof assessments Take hoof photographs Commence treatment
Day 0 – Day 35	Daily treatment of animals in Group 2
Day 7 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take hoof photographs if required
Day 14 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take hoof photographs if required
Day 21 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take hoof photographs if required Farrier undertakes visual hoof assessments
Day 28 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take hoof photographs if required
Day 35 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take photographs of hooves, study completion form Farrier visual hoof assessment and hoof trim
Day 35 (+/- 3 days)	Animal Phase Completed

Table 3: Schedule of Events for the Case Study Arm

Approx. Study Day	Event
Pre-study	Recruit site and cooperators Finalise study plan Receipt of IVP. Receipt of Animal Ethics Committee approval.
Pre-treatment	Screen horses for inclusion criteria Train co-operators
Approx. Day -5	Veterinary examination
Day 0	Veterinarian assesses hoof moisture and undertakes visual hoof assessments Farrier trim hooves, mark coronary band and undertakes hoof visual assessments Take photographs of hooves Commence treatment
Day 0 – Day 84	Twice daily treatment
Day 35 (+/- 3 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take photographs of hooves Farrier assessment and hoof trim
Day 56 (+/- 7 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take photographs of hooves Farrier assessment and hoof trim
Day 84 (+/- 7 days)	Veterinarian measures hoof growth, hoof moisture and undertakes visual hoof assessments Take photographs of hooves Farrier assessment and hoof trim Complete study completion form
Day 84 (+/- 7 days)	Animal Phase Completed

10. STUDY ANIMALS

Animal details will be recorded on the “Animal Information Record”.

Species:	Equine	Number:	Primary Herd Study: 20, Case Study: 1-5 (from an overall number of up to 35 horses)
Breed:	Any breed	Source:	SAHA
Weight:	Any weight	Health & Special Requirements:	<i>Clinically healthy at selection with no systemic illness, vaccinated against Hendra virus, and received no hoof supplements in the previous 5 days to Day 0.</i>
Sex:	Female, male, castrated or a mix		
Age:	Approx. ≥ 2 years, ≤ 20 years		
Method of ID:	Microchip - last 4 digits will be used as Animal ID		

11. ANIMAL MANAGEMENT

11.1 Animal Welfare

Study animals will be managed similarly and with due regard for their welfare. Study animals will be observed according to AEC requirements and a “Record of Animal Care” completed. UNE Animal Ethics approval will be obtained prior to study start.

11.2 Housing & Husbandry

Routine management practices will continue. Horses who commence the study shod will continue to be shod for the study duration. Study animals will be housed either individually or together in paddock(s) with ad lib access to water.

11.3 Health Management

Any routine prophylactic treatments (vaccinations for example) will be given as soon as possible, if necessary, and recorded in the “Animal Information Record” (product name, batch number, expiry date, dose, route and date(s) of administration).

Normal veterinary care and procedures may be performed and will be described in the raw data.

If an injury or illness results in euthanasia or death of a study animal, this will be recorded in the “Study Log” and where possible a post-mortem conducted by a veterinarian. A full “Post Mortem Report”, including the probable cause of death, will be included in the raw data.

11.4 Adverse Events

Any adverse event characterised by the Investigator as IVP related, results in death, is life-threatening, involves a large number of animals, or is a human adverse event, will be recorded on an “Adverse Event Report” form and reported to the Sponsor and AEC. Potential IVP related adverse events includes dermatitis around the coronary band.

11.5 Concurrent Medications

Concurrent medications may be administered for standard management practice and humane reasons, with prior approval from the Investigator, and Sponsor (if relevant). No treatments similar to the IVP will be administered including all topical and oral hoof products. All concurrent medications will be recorded on Form 05 Concurrent Medications Form or “Adverse Event Report”, as applicable, giving identity of materials used (product name, batch number and expiry date), animal ID(s), the reason for use, route of administration, dose and the date(s) administered, and will be included in the raw data and the Study Report.

11.6 Mortality

If an injury or illness results in euthanasia or death of a study animal, this should be recorded in the “Study Log” or “Adverse Event Report”, as applicable. A post-mortem may be conducted, if possible, by a veterinarian and a “Post Mortem Report”, including the probable cause of death, will be included in the raw data.

11.7 Animal Disposal

Animals will remain with SAHA at study completion.

12. STUDY PROCEDURES

12.1 Study Log

All scheduled and unscheduled events during the study should be recorded on the “Study Log”.

12.2 Informed Owner Consent

An “Owner Consent and Livestock Compensation Agreement” form will be required. This form will be signed by the Owner and the Investigator prior to administration of treatment.

12.3 Weather Data

Data from the nearest Bureau of Meteorology weather station for the study period may be included in the raw data.

12.4 Veterinary Exam

At ~Day-5, horses will undergo a routine veterinary exam which will be documented in the raw data on Form 01 – Veterinary Examination.

12.5 Hoof Trimming

Horses involved in the Primary Cohort Herd Study and the Case Study will have their hooves trimmed as per the Schedule of Events. Hoof trimming is a routine husbandry procedure for horses, and will be undertaken by the farrier, and involves the removal of the hoof wall, as well as cleaning, shaping and rasping or smoothing of the edges of the hoof.

12.6 Measure Hoof Growth Rate

Due to the sensitivity of the coronary band, a point distal to the coronary band will be marked on Day 0, and used as the point of measurement. For horses in the Primary Cohort Herd study, this marking will be made on the dorsal wall of the near foreleg hoof, and for horses in the Case study, will be made on the dorsal wall of all four hooves. Hoof wall growth will be measured in millimeters using calipers as per the Schedule of Events. Method of marking will be included in the Final Study Report.

12.7 Measure Moisture Content

For horses in both the Primary Cohort Herd Study, a handheld drywall moisture meter verified prior to first use, and will be used to measure the moisture at the lateral quarter of the dorsal hoof wall (near foreleg for the Primary cohort, and all hooves in the Case study arm) at Day 0, and then as per the Schedule of Events.

12.8 Hoof Structure and General Health Assessment of the Hoof

A Visual Quality Assessment will be made by both a blinded farrier and blinded veterinarian for horses in both the Primary Cohort Herd Study and the Case Study as per the Schedule of Events. The scoring system will be 0 - 4 and will assess the near foreleg in the Primary Cohort, and all four hooves in the Case Study Arm, and will be based on cracks to the hoof, brittleness and shine, as well as coronary band condition, sole quality and flaking and odour, discharge or signs of infection. Photographs of the hooves will also be taken for documentation (medial, lateral and dorsal aspects).

13. ASSESSMENT OF EFFECTS

For horses in the Treatment Group of the Primary Cohort Herd Study, all four hooves will be treated, however, only one hoof (near fore) will be assessed. For horse(s) in the Case Study Arm, all four hooves will be treated and assessed.

13.1 Hoof Growth Rate:

The dorsal hoof wall, distal to the coronary band (of the near foreleg hoof for the Primary cohort, and of all hooves for the case study arm) will be marked on Day 0, and hoof wall growth will be measured in millimetres using calipers as per the Schedule of Events. Growth rate will be compared across treatments and time.

13.2 Moisture Content

Moisture content measurements will be assessed from the lateral quarter of the dorsal hoof wall (of the near foreleg hoof for the Primary cohort, and of all hooves for the case study arm) on Day 0, and then as per the Schedule of Events will be taken. Moisture content will be compared across treatments and time.

13.3 Hoof Structure and General Health Assessment of the Hoof

The Visual Quality Assessments for horses in both the Primary Cohort Herd Study and the Case Study will be compared across horse, treatment & time. Photographs of the hooves will also be taken for documentation.

14. STATISTICAL ANALYSIS

- Primary Cohort:
 - o Growth and moisture compared using t-tests or non-parametric equivalents
 - o Intra-group and inter-group statistical significance assessed ($p < 0.05$)
- Case Study Arm:
 - o Observational data, visual progression, and clinical scoring tracked longitudinally

Other statistical analyses may be conducted as deemed appropriate based on the nature of the data.

Methodology and results will be included in the report.

15. DATA RECORDS

Protocol specifications will supersede facility SOPs. Invetus SOPs will be used as appropriate, and their use will be referenced in the Study Report. Study forms will be sourced as appropriate and included in the raw data.

15.1 Protocol Approval

The Protocol will be approved and signed by all relevant personnel (see page 1) prior to study start.

15.2 Notes to File

Notes to File will be recorded to clarify events or circumstances that may not otherwise be apparent from the raw data. This may include planned or actual changes to the protocol. Notes to File should be communicated to the Sponsor as soon as practically possible.

15.3 Change of Study Personnel

Change of the study Investigator, or other responsible study personnel, will be documented.

15.4 Raw Data

All original raw data pages will be paginated, identified with the study number and signed or initialed and dated by the person making the observation and by the person recording the information.

15.5 Permits

The study detailed in this Protocol is covered by APVMA small trial permit no. PER 7250 .

15.6 Confidentiality

Confidentiality of the raw data, Study Report and results of the study, plus any information received from the Sponsor, will be maintained during and after the study. Publication of material will remain at the sole discretion of the Sponsor.

15.7 Proprietary Rights

This Protocol has been prepared using time, expertise and experience of Invetus Pty Ltd staff and is presented to the Sponsor without obligation. The Protocol and the information contained herein remains the property of Invetus Pty Ltd until such time as the quotation is accepted for carrying out the work described in whole or part. The Sponsor is authorised to use the protocol for internal, scientific and regulatory discussions with other parties for the purposes of determining the correctness or otherwise of the Protocol, but is not authorised to use the Protocol for any other purpose including the obtaining of alternative quotations without the written consent of Invetus Pty Ltd. This restriction may be removed by the purchase of the Protocol for an agreed fee.

16. STUDY REPORT

A Study Report will be prepared by the Investigator, or designee. The Study Report will follow the current Invetus template. Data listings of each variable measured will be included.

The original signed Study Report with the original raw data, and Statistical Analysis will be submitted to the Sponsor. A copy of the Study Report, plus appendices, will be archived as detailed below.

17. DOCUMENT ARCHIVING

Raw data generated during the animal phase of this study will include, but is not limited to, the study forms listed in Appendix 03. A copy of the raw data, plus the documentation listed below, will be archived at Invetus Pty Ltd, Clarks Road, Armidale, NSW, Australia for a minimum period of six years, according to relevant SOPs:

- Final Study Report, plus any Notes to File
- Statistical Analysis
- Raw data generated to perform statistical analyses of study data
- Final Protocol
- Animal Ethics Authority
- Record of Animal Care
- Safety Data Sheet(s)
- Weather data
- Relevant study related correspondence

18. REFERENCES

1. Holzauer, M., Bremer, R., Santman-Berends, I., Smink, O., Janssens, I. and Back, W., Cross-sectional study of the prevalence of and risk factors for hoof disorders in horses in the Netherlands, Preventative Veterinary Medicine, 01 May 2017, Vol 140, Pages 53-59, <https://www.sciencedirect.com/science/article/abs/pii/S0167587716304639>
2. Thirkell, J. and Hyland, R., A Preliminary Review of Equine Hoof Management and the Client-Farrier Relationship in the United Kingdom, Journal of Equine Veterinary Science, December 2017, Vol 59, Pages 88-94, <https://www.sciencedirect.com/science/article/abs/pii/S0737080617303659>

19. APPENDICES

Appendix 01 Glossary of Abbreviations
Appendix 02 Standard Operating Procedures
Appendix 03 Study Forms
Appendix 04 Horse Body Condition Score Chart

Appendix 01 Glossary of Abbreviations

Abbreviation	Definition
AE	Adverse Event
AEC	Animal Ethics Committee
~	Approximately
ARA	Animal Research Authority
°C	Degrees Celsius
%	Percentage
APVMA	Australian Pesticides and Veterinary Medicines Authority
BW	Body Weight
CP	Control Product
GSP	Good Scientific Practice
GL	Guideline
ID	Identification
IVP	Investigational Veterinary Product
kg	Kilogram(s)
L	Litre(s)
g	Gram(s)
µg	Microgram(s)
mg	Milligram(s)
mL	Millilitre(s)
mm	Millimeter(s)
N/A	Not Applicable
No.	Number
NSW	New South Wales
Pty Ltd	Proprietary Limited
QA	Quality Assurance
R&D	Research and Development
SDS	Safety Data Sheet
SMF	Study Master File
SOP	Standard Operating Procedure
VICH	Veterinary International Conference on Harmonisation
WHP	Withholding Period

Appendix 02 Standard Operating Procedures

SOP No.
STU-308

SOP Title
Test Item and Reference Item Management

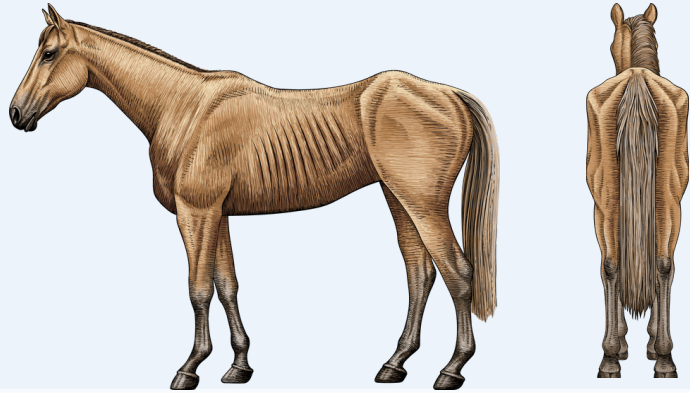
Appendix 03 Study Forms

Study forms may be added or amended during the study without the need for a Protocol Amendment or Deviation.

Form No.	Form Title
Form 01 (FLD-409.02)	Veterinary Examination – Single Animal
Form 02	Veterinarian Assessments
Form 03	Animal treatment Record
Form 04	Farrier Assessments
Form 05	Concurrent Medication Form
Form 06	Group Allocation
STU-304.01	Owner Consent & Livestock Compensation Agreement
STU-304.05	Animal Accountability
STU 304.06	Consent to Use Personal Data
STU-308.01	Test or Reference Item Register
STU-308.02	Test or Reference Item Accountability
STU-308.03	Drug Reconciliation
STU-309.07	Study Log
STU-309.08	Signature Log
STU 323.03	Note to File
FLD-409.04	Post Mortem Report
FLD-409.05	Adverse Event Report

Appendix 04 Body Condition Scoring - Horses

Body Condition Scoring – Horses



1 Emaciated

Extremely emaciated with very poor body condition

Neck: Noticeable bone structure.

Withers: Noticeable bone structure.

Back: Spinous processes project prominently.

Tailhead & Hookbones: Project prominently.

Ribs: Project prominently.

Shoulders: Noticeable bone structure.

2 Very Thin

Very thin and emaciated with a slight fat covering over base of spinous processes

Neck: Faintly discernible.

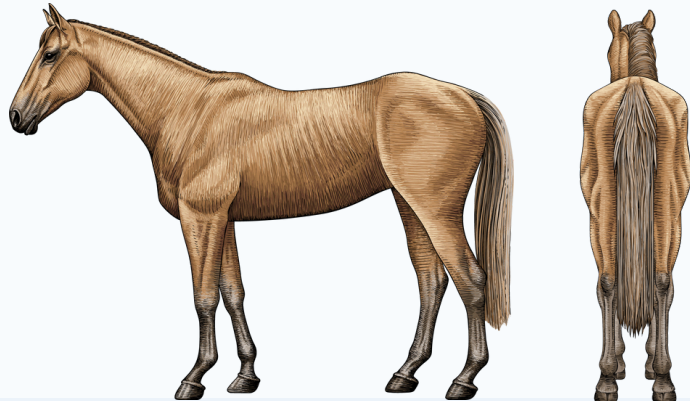
Withers: Noticeable bone structure.

Back: Transverse processes of lumbar vertebrae feel rounded; Spinous processes project prominently.

Tailhead: Prominent.

Ribs: Prominent.

Shoulders: Faintly discernible.



3 Thin

Neck: Accentuated.

Withers: Accentuated.

Back: Fat buildup halfway on spinous processes but easily discernible; Cannot feel transverse processes.

Tailhead: Prominent but individual vertebrae cannot be felt; Hookbones appear round and are discernible. Pin bones are not distinguishable.

Ribs: Discernible with light fat cover over the ribs.

Shoulders: Accentuated.

4 Moderately Thin

Neck: Not overtly thin.

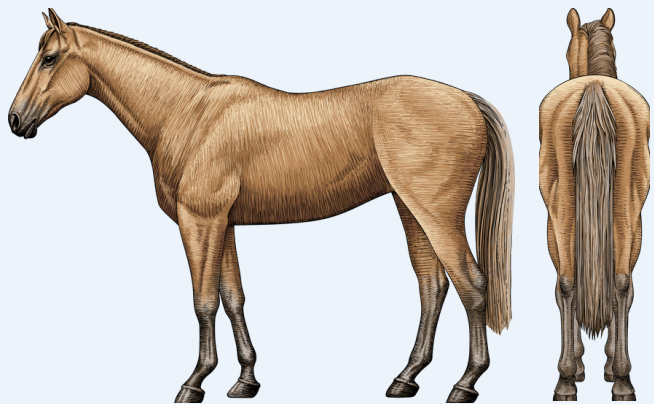
Withers: Not overtly thin.

Back: Slight crease along the back.

Tailhead: Hookbones not discernible; fat can be felt, but prominence varies between horses.

Ribs: Faint outline discernible.

Shoulders: Accentuated.



5 Moderate or Ideal

Neck: The back is flat with no ridge or crease.

Withers: Rounded over spinous processes.

Back: The back is level.

Tailhead: Area feels spongy.

Ribs: Easily felt but not visible.

Shoulder: Blends smoothly into the body.

6 Fleshy

Neck: Some fat accumulation.

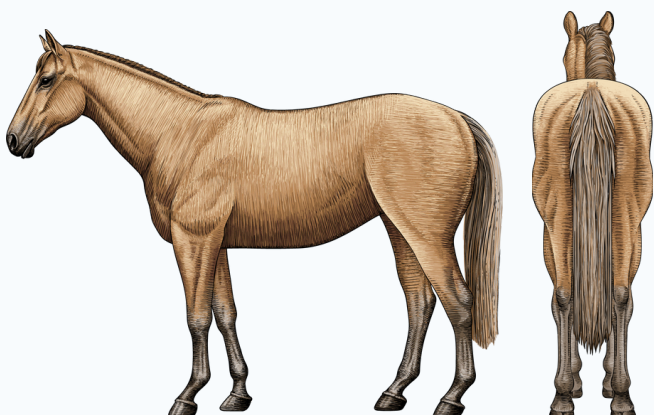
Withers: Some fat accumulation.

Back: May have a slight positive crease along back.

Tailhead: Area feels soft.

Ribs: Area over the ribs feels spongy.

Shoulders: Some fat accumulation.



7 Overweight

Neck: Fat accumulation.

Withers: Fat accumulation.

Back: May have a positive crease along the back.

Tailhead: Feels soft.

Ribs: Individual ribs can be felt; Noticeable filling between the ribs with fat.

Shoulders: Fat accumulation.

8 Obesity

Neck: Noticeable thickening.

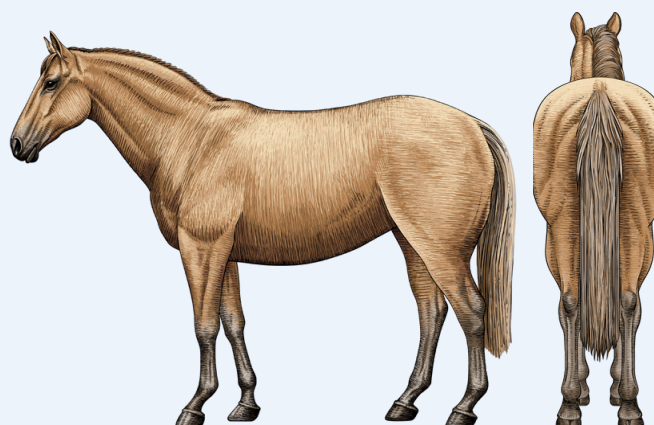
Withers: Area filled with fat.

Back: Positive crease along the back

Tailhead: Feels very soft.

Ribs: Difficult to feel.

Shoulders: Area is filled in, flush with the body.



9 Severe Obesity

The horse has a very rounded appearance with pronounced fat deposits.

Neck: Bulging fat.

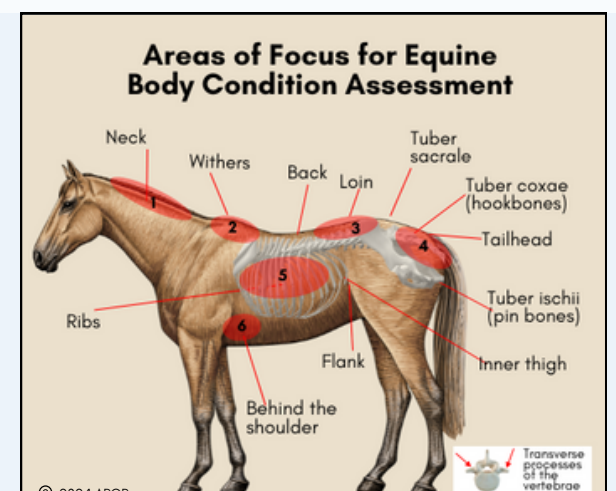
Withers: Bulging fat.

Back: Positive crease down the back

Tailhead: Bulging fat.

Ribs: Patchy fat over the ribs.

Shoulders: Bulging fat.



A body condition score (BCS) is a tool used to assess body fat, potential nutritional imbalances, and overall health. Other helpful tools for Equine include Cresty Neck Scoring, an assessment tool used to visually evaluate the amount of fat deposited along a horse's neck associated with identifying horses at risk for metabolic disorders and a Muscle Condition Score to help identify areas of muscle loss or imbalance.

Henneke, D. R., Bellows, R. A., & Cole, J. B. (1983). Relationship between condition score, physical measurements, and body fat percentage in mares. *Equine Veterinary Journal*, 15(4), 371-372.

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