Biological sample collection and handling methods for fat-soluble vitamin and trace mineral analysis

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Summary

Diagnostic reports of biological samples submitted from farms are essential to correctly identify any underlying issues in a herd, including disease and improper nutrition. Proper sample collection, handling, and storage are critical to most accurately diagnose health complications or nutritional status. When possible, sample pigs before they eat, keep tissue samples frozen, avoid hemolyzed blood samples, and minimize transport time to the diagnostic laboratory. Concerns regarding sample collection and storage can be addressed with a veterinary diagnostic laboratory.

Keywords: swine, vitamin, sampling, pre-analytical

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Resumen - Métodos de colecta y manipulación de muestras biológicas para el análisis de vitaminas liposolubles y minerales traza

Los reportes de diagnóstico de las muestras biológicas enviadas desde las granjas son esenciales para identificar correctamente cualquier problema importante en una piara, incluidas las enfermedades y la nutrición inadecuada. La recolección, el manejo, y el almacenamiento adecuados de las muestras son fundamentales para diagnosticar con mayor precisión las complicaciones de salud o el estado nutricional. Cuando sea posible, tome las muestras de los cerdos antes de que coman, mantenga las muestras de los tejidos congeladas, evite las muestras de sangre hemolizada, y minimice el tiempo de transporte al laboratorio de diagnóstico. Las dudas relacionadas con la recolección y el almacenamiento de muestras se pueden abordar con un laboratorio de diagnóstico veterinario.

Résumé - Méthodes de collecte et de manipulation d'échantillons biologiques pour l'analyse des vitamines liposolubles et des oligo-éléments

Les rapports de diagnostic des échantillons biologiques soumis par les fermes sont essentiels pour identifier correctement tout problème sous-jacent dans un troupeau, y compris les maladies et une mauvaise alimentation. La collecte, la manipulation, et l'entreposage appropriés des échantillons sont essentiels pour diagnostiquer avec le plus de précision possible les complications de santé ou l'état nutritionnel. Dans la mesure du possible, il faut échantillonner les porcs avant qu'ils ne mangent, conserver les échantillons de tissus congelés, éviter les échantillons de sang hémolysé, et minimiser le temps de transport vers le laboratoire de diagnostic. Les préoccupations concernant la collecte et l'entreposage des échantillons peuvent être discutées avec le personnel d'un laboratoire de diagnostic vétérinaire.

Sample collection is a crucial component of research and diagnostics. Sample analysis can help determine the root cause of disease issues or the nutritional status of the animal. When evaluating nutritional status, it is essential that samples are collected, handled, and stored appropriately so that nutrient analysis is accurate. Incorrect sample handling may result in misleading analytical results ranging from false deficiencies to toxicities. Specifically, factors that can influence the vitamin analysis of samples include blood tube type, time of collection after a meal, hemolysis, storage, and the animal sampled.

Blood tube type

Blood tube types differ depending on whether the collection is for serum, plasma, or if a specific coagulation method is desired (Table 1). Serum is the liquid portion of the blood that remains after the blood is allowed to clot and then centrifuged. Plasma is the liquid portion centrifuged from unclotted blood. The serum fraction will have lower protein concentrations and a lower number of platelets, erythrocytes, and leukocytes than plasma.[2] It is imperative that samples meant to provide plasma are not allowed to clot because the clotting process will utilize proteins in the sample which will lower the protein content,[2] thus compromising the analytical results of the sample. To avoid plasma samples clotting, collect the blood in a timely manner, and immediately invert the blood tube 8 to 10 times to properly distribute the anticoagulant additives throughout the sample. Serum blood

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Table 1: Blood tube types and functions*

Blood tube top color	Additive	Mode of action	Sample type and considerations
Solid red or red tiger top	Silicon coated sides; red tiger top will have a gel separator	Clot activator will ensure that blood clots in a timely manner	Serum determinations, such as vitamins and other chemistries
Purple/lavender	EDTA	Forms insoluble calcium salts and will prevent clot formation	Hematology and immunohematology
Green	Sodium or lithium heparin	Antithrombin and anti-thromboplactin prevent clot formation	Plasma determination and blood gas analysis
Light blue	3.2% sodium citrate	Forms the insoluble salt calcium citrate and will prevent clot formation	Coaguation determination and platelet function
Grey	Sodium fluoride, and sodium or potassium oxalate	Forms the insoluble salt calcium oxalte and prevents clot formation	Glucose determinations
Royal blue (red stripe)	No preservative	Clot activator will ensure that blood clots in a timely manner	Trace element, toxicology, and nutritional testing
Royal blue (purple/lavender stripe)	Potassium EDTA	Prevents clot formation	Trace element, toxicology, and nutritional chemistry analysis

* Table adapted from Benjamin[1] and BD Vacutainer.[5]

tubes (red, red tiger stripes, or gold tops) allow the collected blood to clot, while plasma (purple or green tops) contain additives, such as sodium or lithium heparin, that prevent blood from clotting.[1]

Using serum versus plasma tubes can affect the analysis of the nutrient in question.[3] For example, Elefson and Greiner[3] showed that vitamin D (25-hydroxyvitamin D_3 metabolite) concentrations were significantly lower when using plasma tubes compared to serum tubes (31 ng/mL vs 34 ng/mL, respectively). Furthermore, it was found that gilts had lower vitamin D metabolites than barrows,[3] which could be due to sexual maturity. Reporting which blood tube was used to the diagnostic laboratories will help to ensure proper reports and interpretations made for the conducted analysis. Additionally, care should be taken when collecting blood for trace minerals, as many components of blood tubes are made with minerals, such as zinc, which can lead to misleading results.[4] Royal blue topped blood tubes are made specifically for either serum or plasma mineral analysis.[5]

Time of collection

In general, pigs have *ad libitum* access to feed. However, there are situations, such as with sows and boars, when pigs are limit fed to help maintain an ideal body condition.[6] When pigs are limit fed, there is a potential for a bolus of nutrients to pass through the bloodstream resulting in nutrient spikes.[3] However, digestion, absorption, nutrient interaction with transporters during absorption, and redistribution of nutrients occur at different rates depending on the nutrient in question. For example, iron and copper have been known to compete for epithelial transporters, thus affecting how much of the other is absorbed.[7] Additionally, the form in which a nutrient is fed to an animal can also affect absorption. For instance, it has been reported that free amino acids are absorbed more quickly than protein-bound amino acids.[8-10] Faster absorption results in the free amino acids appearing in circulation faster than the protein-bound amino acids. Thus, it is best to take blood samples before a given meal to avoid any potential peaks in nutrients in limit-fed animals.

Hemolysis

Hemolysis occurs when red blood cells lyse and their contents interact with the serum or plasma. Hemolysis often occurs post blood draw when shear force is applied to the blood sample. For example, when a blood sample is taken via a syringe and then transferred to a blood tube through the needle, the red blood cells are subjected to a shear force. Additionally, freeze-thaw cycles can also cause hemolysis,[11] and thus freeze-thaw cycles should be avoided. When the contents of red blood cells are released, the serum or plasma components change, such as increasing levels of zinc and iron[12] and altered results for sodium, potassium, and phosphorous.[13] Furthermore, it has been documented that both iron and zinc can form complexes with vitamin A, thus impacting the measured vitamin A concentration within the body.[14,15] It has been shown that hemolysis can decrease vitamin A (retinol) concentration in plasma samples.[3] Additionally, vitamin E (alpha-tocopherol) concentration has been shown to decrease due to red blood cell hemolysis.[16]

Storage

The standard storage method for biological samples is to process and freeze samples as quickly as possible once collected and keep the sample frozen until the time of analysis. Keeping samples frozen helps to prevent any degradation that might occur to the organic components,

such as vitamins.[17] It is common in the United States for samples collected on farm to be processed and submitted frozen in a cooler with ice packs or dry ice, which is shipped overnight to the diagnostic laboratory of choice. However, weather or human error can result in a delay of sample reception at the diagnostic laboratory. The longer samples are not frozen, the greater the chance for an altered vitamin status to occur. Recent work by Elefson and Greiner[3] showed that samples can be stored in a Styrofoam cooler with ice packs for 2 days without significant change to vitamins A (retinol) and E (alpha-tocopherol) in serum and liver.

Animals sampled

If a nutritional issue is suspected on a farm, biological samples provide information regarding nutritional status of the herd. However, sampling only unhealthy animals may not reflect the herd's nutritional status as the animal may not have been consuming food for an extended period. Animals will mobilize reserves to preserve homeostasis. For example, if a diet is deficient in vitamin A, liver vitamin A reserves are mobilized to keep circulating vitamin A levels constant.[17] Furthermore, this mobilization from the liver will result in lower liver vitamin A levels in an ill animal that is not eating compared to healthy animals, regardless of vitamin A concentration in the feed. There may also be sex differences for some nutrients[3] that will also influence how diagnostic results can be interpreted.

Depending upon the suspected deficiency or toxicity, the veterinarian may need to collect samples from both affected and unaffected animals, as unaffected individuals offer a comparative baseline. In addition, information on the nutrient composition of the diet should be provided. Identifying and understanding individual nutrient levels of the pigs within a herd is critical due to the variation from farm to farm in diets fed as well as pig age, health status, and environment.

Feed samples

There are many factors to consider when taking a feed sample to optimize the sample being representative of what the animal is consuming. Factors include size of the sample collected, equipment used for the collection, location of sample collection, and sample storage. Collecting a large feed sample will increase the likelihood that the sample is representative of the batch of feed that is being mixed. Feed samples from different rations or mixed batches should never be pooled so that any nutritional issues cannot be correctly linked back to the source. A hand-grab collection or the use of a probe are common ways to collect feed from either a feeder or mixer.[18] Samples collected with a probe have been documented to have less variability.[19] When using a probe to collect a feed sample, the probe should be able to reach the bottom of the bulk carrier where the feed is located.[18] Furthermore, at least 10 samples from 10 evenly-spaced locations in the bulk carrier should be collected to ensure an accurate representation of the feed.[18] Collection of feed should never be based on ease of collection (ie, side of grain bin where grain is not actively flowing for milling and animal consumption). Additionally, a sample of the premix should be collected along with a complete feed sample to ensure accurate analysis of the premix. For example, vitamins have low inclusion levels in the diet resulting in greater incidence of vitamin-level variation when testing complete feed samples compared to vitamin premixes. Vitamins in feed have different sensitivities to temperature, humidity, and light, with fat-soluble vitamins being some of the most senstive.[20] Thus, after a sample is collected, it should be stored in a cool and dark location to help prevent degradation of organic compounds in the feed. More information on collecting feed samples can be found on the Iowa Pork Industry Center website[21] and the Kansas State University Animal Science and Industry website.[18]

Recommended Sample Process

Knowing how much sample is needed and what type of sample should be collected is essential to help diagnose any disease or nutritional issues. Questions about collecting samples should be directed to a veterinary diagnostic laboratory. In general, there are a few key points to remember when a sample is being collected (Table 2). If a plasma sample is being collected, ensure blood is collected in the blood tube in a timely manner, and the blood tube is inverted to mix the anticoagulant to prevent clotting. To avoid hemolysis in blood samples, do not force blood through a needle and syringe into a blood tube. Instead, a blood collection needle attached to a vacutainer hub allowing for direct collection of blood is ideal, as the vacuum of the tube is such that it provides a constant flow rate that prevents shearing of blood cells. The time of sample collection should be noted if sampled animals are limit fed and samples should be collected before the animal is fed to avoid any nutrient spikes. Samples should be processed and frozen as quickly as possible. Processing samples includes centrifuging blood samples so that plasma or serum can be aliquoted off from red blood cells prior to being frozen. If samples are being shipped to the diagnostic laboratory, then ice packs should be included in the cooler with the samples to keep samples as cool as possible. A sample from healthy animals and ill animals is critical to know what is expected within the herd in question so that a diagnosis is easier to determine for unhealthy animals.

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Conflict of interest

None reported.

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Table 2: Key concepts of sample collection

Topic	Take-home point
Blood	There are different components of blood, such as serum or plasma, that will need to be isolated based on the analysis in question.
Blood tube type	The type of blood tube used will help to isolate either serum or plasma. Certain blood tube types are used for specific analysis over others. Consult with a veterinary diagnostic laboratory to confirm which blood tube would be best to use.
Timing of collection	For pigs that are limit fed, there could be nutrient spikes in blood samples from the nutrients being redistributed to the peripheral tissues after meal consumption.
Hemolysis	When red blood cells reputure, their contents are released and can interfere with the nutrient analysis of a blood sample. It is best to avoid hemolysis by avoiding freeze-thaw cyles and shear force being applied to the sample.
Storage	All samples should be processed, including centrifugation, and frozen as quickly as possible to help prevent any degradation.
Animals being sampled	Both healthy and ill animals should be sampled to help take into consideration what "normal" is on a farm and how the sick animal compares.
Feed samples	A large feed sample should be collected from multiple locations in the mixer or feeder and stored in a cool and dark location to help ensure the analyzed feed is an accurate representation of the feed that is being consumed. Sampling the premix in addition to complete feed can help provide information on nutrients included in a small quantity.

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