

Method Needs and Fitness for Purpose Statement – Final

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Project: Determination of vitamin D in animal feeding stuffs

Project Leader:

Project Team:

1.0 Needs¹:

Vitamin D is a fat soluble “vitamin”, essential for normal bone formation in animals; it is essential in the diets of animals raised with insufficient exposure to sunlight. Adequate sunlight results in the production of sufficient vitamin D₃ from 7-dehydrocholesterol in the skin, so Vitamin D can be considered a vitamin only in the sense that, under modern farming conditions, many animals are raised in total confinement with little or no exposure to natural sunlight. Lack of adequate photoproduction of vitamin D₃ or inadequate dietary supplementation of vitamin D in the diet leads to failure of the bones to calcify normally. This metabolic disease is known as rickets in the young and as osteomalacia in adults. Once this deficiency was recognized, the dietary supplementation of vitamin D became a commonly accepted nutritional practice. The dietary requirements for most animal species are in the range of 200 to 1200 IU/kg (90 to 600 IU/lb) of diet. The vitamin D sterols that are used in animal feed and their relative biological potencies are listed below. The method should separate the following and quantitate those with an asterisk.

Form	Relative Potency
*Vitamin D ₃ (Cholecalciferol – animal source)	1
*Vitamin D ₂ (Ergocalciferol – plant source)	<1 variable by species
Dihyrotachysterol (DHT)	0.05-0.1
*25-OH-D ₃ (Calcidiol)	2-5
1,25-(OH) ₂ -D ₃ (Calcitriol)	5-10
αOH-d ₂ (αCalcidiol)	5-10
αOH-D ₃	5-10

*Need to quantitate

1 international or USP Unit of vitamin D activity is defined as the activity of 0.025 µg of vitamin D₃ contained in the USP vitamin D reference standard. For poultry, the term *international chick unit* (ICU) is employed with reference to the use of D₃ versus D₂. D₂ is not utilized by chickens and other birds as the nutritional precursor to the hormone to promote calcium absorption and bone and eggshell development.

There are two current AOACI Methods: AOAC 936.14 and AOAC 982.29. AOAC 936.14 is a bioassay that involves feeding young chicks a rachitogenic diet for 21 days and determining the ash content of dry fat free tibia from both the chicks fed the rachitogenic diet and those fed known amounts of vitamin D reference standard. This method is not widely used in laboratories testing feeds. AOAC 982.29 is a LC method that involves an elaborate cleanup on alumina column followed by LC on a normal phase column and UV detection. The LC

method is laborious, difficult and is also not in widespread use in feed laboratories. An improved LC method, preferably reverse phase, is desired employing improved clean up and sensitivity is desired to detect the forms of vitamin D commonly added to animal feeds.

The developed methodology should cover a large range of vitamin D levels and should be applicable to all types of feeds to which it is incorporated (for example: dry feeds, vitamin mineral mixes, milk replacer, liquid supplements, dry and wet pet food) for a wide variety of animal species, including farm and companion animals. The method should produce results comparable to the current AOACI OMA (982.29), or better, and be more acceptable to feed laboratories.

1.1 Performance Needs:

Accuracy: (See Recovery)

Feed premixes: 90 – 110 %

Feeds and feed ingredients: 70 – 130 %

Applicability:

D3 concentration depends on species and dietary inclusion rates. Typical ranges are as follows:

Complete feeds – 100 to 5,000 IU/lb (220 to 11,000 IU/kg)

Premixes – 20,000 to 2,000,000 IU/lb (44,000 to 4,400,000 IU/kg)

Base mixes – 4,000 to 50,000 IU/lb (8,800 to 110,000 IU/kg)

Concentrates – 1,000 to 25,000 IU/lb (2,200 to 55,000 IU/kg)

Dairy/Beef – 5,000 to 200,000 IU/lb (11,000 to 440,000 IU/kg)

Detection Limits:

Feeds, feed premixes, and feed ingredients: 30 IU/lb (66 IU/kg)

Determination Limits:

Feeds, feed premixes, and feed ingredients: 100 IU/lb (220 IU/kg)

Precision Repeatability:

> 10,000 IU/lb (> 22,000 IU/kg): $CV_r =$ or < 10 %

≤ 10,000 IU/lb. (≤ 22,000 IU/kg): $CV_r =$ or < 15 %.

Precision Reproducibility:

> 10,000 IU/lb (> 22,000 IU/kg): $CV_R =$ or < 20 %

≤ 10,000 IU/lb (≤ 22,000 IU/kg): $CV_R =$ or < 30 %.

Range:

100 – 2,000,000 IU/lb (220 to 4,400,000 IU/kg)

Recovery:

Feed premixes and base mixes: 80 – 115 %

Feeds and feed ingredients: 70 – 125 %

Selectivity:

The method is to be free of interferences from the matrix, other drugs, vitamins, and minerals.

Linearity of standard curve:

$r \geq 0.999$ and 95% confidence limits of the y-intercept must include zero.

Special Considerations:

Performance of this method should be comparable to AOACI OMA 982.29.

The method is to be rugged/robust and critical parameters are to be identified and controlled.

Methods must be capable of handling encapsulation of the vitamin prior to incorporation into animal feed.

Methods should consider the use of internal standards.

Method performance criteria are to be defined. Familiarization plan is to be suggested which will demonstrate that the laboratory analyst can capably perform the method prior to analyzing samples.

Quality control plan is to be suggested along with warning and out of control limits.

Traceability:

Acceptable analytical standard material is available from U.S. Pharmacopeia (USP).

Comments

While not necessary, a highly desirable method would combine the determination of vitamins A, D and E.

Method Performance:

Fitness for Purpose Review

Fitness for Purpose Statement

¹ *Vitamin Tolerances of Animals* (1987), The National Academies Press.