# EFFECT OF PELLETING AND EXPANDING PROCESSES ON VITAMIN A STABILITY IN ANIMAL FEEDS

# UTICAJ PELETIRANJA I EKSPANDIRANJA NA STABILNOST VITAMINA A U HRANI ZA ŽIVOTINJE

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# ABSTRACT

The stability of vitamin A (retinol-acetate) in pelleted feed for broilers and expanded feed for piglets was controlled during the storage under extreme conditions (at temperature 60°C and relative humidity 80%) in a period of three months. Determination of vitamin content was performed by High-Performance Liquid Chromatography (HPLC). The concentrations of vitamin A in the untreated samples of feed for broilers and piglets decreased during the three month storage period to 60% and 70% of their initial values, respectively. In the samples of pelleted feed for broilers and expanded feed for piglets, the concentrations of vitamin A decreased to 39% and 50% of their initial values, respectively. Pelleting and expanding processes had significant effects on vitamin A stability in animal feeds. Losses, in general, were in the range found in other studies.

Key words: feed processing, stability, vitamin A, high-performance liquid chromatography.

# REZIME

Vitamini, kao biološki aktivne komponente, prilično su osetljivi na fizičke i hemijske uticaje. Procesi koji se primenjuju pri proizvodnji hrane za životinje teže da poboljšaju distribuciju hranljivih materija i svarljivost ugljenih hidrata. Međutim, ovi procesi utiču na stabilnost pojedinih nutrijenata, kao što su vitamini, koji se lako oksiduju. Nekoliko faktora utiče na stabilnost vitamina u procesima peletiranja, ekspandiranja i skladištenja, a to su vlažnost, temperatura, vreme kondicioniranja, oksido-redukcione reakcije, pritisak, trenje i svetlost.

U radu je ispitana stabilnost vitamina A (retinol acetat) u peletiranoj hrani za brojlere i ekspandiranoj hrani za prasad, u kontrolisanim, ali ekstremnim uslovima relativne vlažnosti (80%) i temperature (60°C), tokom tri meseca. Određivanje sadržaja vitamina izvršeno je tečnom hromatografijom pod visokim pritiskom. Tokom tri meseca skladištenja, u netretiranim uzorcima hrane za brojlere, sadržaj vitamina A je opao do 60% od početne vrednosti, dok je u netretiranim uzorcima hrane za prasad njegov sadržaj opao do 70% od početne vrednosti. U uzorcima peletirane hrane za brojlere, tokom tri meseca skladištenja, sadržaj A vitamina je opao do 39% od početne vrednosti, dok je u uzorcima ekspandirane hrane za prasad smanjen do 50% od početne vrednosti. Na osnovu dobijenih rezultata zapaženo je da procesi peletiranja i ekspandiranja imaju značajan uticaj na stabilnost vitamina A u hrani za životinje. Gubitak u sadržaju vitamina A, uglavnom je u granicama istraživanja drugih autora.

Ključne reči: peletiranje, ekspandiranje, stabilnost, vitamin A, tečna hromatografija pod visokim pritiskom.

# **INTRODUCTION**

Feed technology has advanced in the past two decades. However, NRC (1994) vitamin requirements for the monogastric animals have not changed to any great extent due to limited research in this area. Conditioning temperatures have increased in order to reduce microbial contamination and improve pellet quality and/or production rate. From a practical standpoint, vitamin activity may be decreased in finished feeds due to heightened conditioning parameters. Heat, friction, steam (moisture), pressure, and oxidation are some factors that influence vitamin stability during feed processing (Anderson and Sunderland, 2002; Sredanović et al., 2003). Gadient and Fenster (1994) reported that moisture addition influences vitamin activity more than conditioning temperature. Moisture softens the coating of vitamins allowing oxygen and other compounds access, thus creating vitamin destruction by accentuating chemical reactions. Oxidation-reduction reactions are a primary concern with vitamin activity. It decreases vitamin stability by adding or removing hydrogen atoms to double bonds and/or hydroxyl groups of vitamins

Pellet conditioners, expanders, and extruders are the three basic types of processing equipment used in the feed production

industry. Pellet mill conditioners are used to add steam prior to pelleting (*Vukmirović et al., 2010*). The addition of steam increases temperature of the feed mash in the range of 85 to 100°C for approximately 20 seconds. Extended pellet mill conditioners enable the feed to be subjected to the increased temperature for as long as 20 minutes, depending on the length of the conditioner. The increase in conditioning time allows for increased starch gelatinization, protein denaturation, pathogen reduction, improved pellet quality, and increased production rate (*Sredanović et al., 2005*).

These processes are intended to increase the overall quality of feed ingredients. For example, the digestibility may be increased or the palatability improved (*Riaz, 2007*). On the other hand, micro ingredients such as vitamins are more likely to be damaged by the feed manufacturing process. Vitamin bioavailability is affected by the stability of the vitamin and the utilization efficiency (*Baker, 1995*). This is way special attention must be paid when applying thermal processes for feed production.

Vitamins are important for livestock nutrition and growth. They are intentionally added to animal feed in order to achieve the improvement of health and growth performance of animals and the characteristics of products of animal origin. Additionally, if absent from the diet, vitamins can cause a specific deficiency disease because they are necessary for normal metabolism.

In this study, the stability of formulated commercial form of retinol acetate (vitamin A) was evaluated in pelleted feed for broilers and expanded feed for piglets, in order to determine what the losses of vitamin A are after thermal processing of animal feeds.

## MATERIAL AND METHOD

#### Samples

Four samples of animal feed were examined:

1. Complete mixture for broilers I (untreated) - UTB

2. Pelleted feed for broilers: - PEB

*Pelleting conditions:* Complete mixture was conditioned in double-shaft steam conditioner Muyang SLHSJ0.2A (China), until material reached temperature of 80°C, with direct water addition into feed mash during conditioning. Material moisture content after conditioning process was 15.5%.

The material was pelleted on a flat die pellet press 14-175, AMANDUS KAHL GmbH & Co. KG (Germany). A die with 6 mm diameter of the openings and with press way of 36 mm was used (diameter to length ratio 1:6). The pellets were collected at pelleting temperature of 60°C. The rate of product flow was 18.6 kg/h. After pelleting, pellets were stored for 24 hours under room conditions in order to achieve stabile temperature and subsequently milled by hummer mill with sieve opening of 4 mm.

3. Complete mixture for piglets II (untreated) – UTP

4. Expanded feed for piglets: - EXP

*Expanding conditions:* Complete mixture was conditioned in double-shaft steam conditioner Muyang SLHSJ0.2A (China), until material reached temperature of 80°C, with direct water addition into feed mash during conditioning. Material moisture content after conditioning process was 25.50%.

A single screw annular gap expander (OEE 8, AMANDUS KAHL GmbH & Co. KG, Germany) with a length-to-diameter ratio of 8.5 : 1.0 and capacity of 100 kg/h was used for obtaining expanded product at  $130 \pm 1^{\circ}$ C. The speed of passage of material was 15.84 kg/h. The products were stored for 24 hours under room conditions in order to achieve stabile temperature and afterwards milled by hummer mill with sieve opening of 4 mm.

One kilogram of each feed for broilers contained minimum 12000 IU/kg of vitamin A and each sample of feed for piglets II contained minimum 15000 IU/kg of vitamin A. Vitamin A contained in the samples was in the form of retinol acetate. Samples were stored in glass bottles (370 ml volume) in the Climate chamber Binder KBF series (E5.2), (Binder GmbH, Tuttlingen, Nemačka), in the dark at the temperature of 60°C and at the relative humidity of 80%, and analysed at the beginning of the study and after each week, a total of three months (12 weeks).

### **Analytical procedure**

The standard solution of vitamin A (Retinol acetate, analytical standard min. 98.6%, Lot. No.: LB87725V, Supelco analytical, USA) with the concentration of approximately 3000 IU/ml were prepared in 2-propanol. This stock solution was used to prepare a series of standard solutions for calibration curve.

The preparation of samples (extraction procedure) for the determination of vitamin A was done according to the official methods of the Association of Official Analytical Chemists (*AOAC*, *1995*).

Chromatographic separations were performed on a 2,1 x 100 mm Zorbax eclipse plus  $C_{18}$  (Agilent, USA) 1.8  $\mu$ m HPLC column. Methanol was used as mobile phase at a flow-rate of 0.3-

ml/min and a pressure of 230 Bars. The injection volume was  $5-\mu l$  and UV detection was performed at the wavelength of 326 nm on Agilent, USA photodiode array UV-visible detector. All quantization was determined by peak area using an Agilent integrator. Based on the established chromatographic conditions, repeated injections of standard solutions were made 10 times onto the HPLC system.

### **Statistical procedure**

In order to compare means, the independent samples *t*-test of the STATISTICA Version 10 (2010) statistical software package was used.

### **RESULTS AND DISCUSSION**

Thermal processing (pelleting, roasting, expansion, and extrusion) of animal feed represents methods frequently used in order to improve growth rate, efficiency of gain, nutrient stability, and facilitate feed handling (*Fairfield, 2003*). Thermal processing has been shown to reduce effectiveness of functional protein ingredients such as enzymes and less stable nutrients such as vitamins (*Dozier, 2002*).

The results of vitamin A determination in the investigated samples of animal feed are presented in Table 1.

At the beginning of the study, the initial concentration of vitamin A in the tested samples of complete mixture for broilers and complete mixture for piglets were within the range prescribed by the manufacturer (minimum 12000 IU/kg of vitamin A in feed for broilers and minimum 15000 IU/kg of vitamin A in feed for piglets). However, at the beginning of the study, the concentration of vitamin A in pelleted feed for broilers decreased to 93% of its initial values while its average concentration in the sample of expanded feed for piglets decreased to 97%.

Table 1. The average concentration of vitamin A in investigated feed samples. IU/kg

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Feed	Beginning		2 Months	3 Months
	of the study			
UTB	13600±400 <sup>a</sup>	11300±400 <sup>ab</sup>	$10100 \pm 200^{\circ}$	$8200 \pm 400^{e}$
PEB	12700±200 <sup>a</sup>	$12400 \pm 200^{d}$	9900±300 <sup>c</sup>	$8890 \pm 200^{d}$
		$13600 \pm 300^{\circ}$		
EXP	$15800 \pm 100^{a}$	$13600 \pm 400^{b}$	10700±200°	$7900 \pm 300^{d}$

UTB- complete mixture for broilers (untreated); PEB- pelleted feed for broilers; UTP- complete mixture for piglets (untreated); EXP- expanded feed for piglets; Values concerning the vitamin A content in samples with different superscript letters (ae) differ significantly (P < 0.05)

The observed increase in vitamin A content, between pelleted and expanded feed samples, was not statistically significant (P > 0.05), however, it shows that the pelleting process has a greater impact on the degradation of vitamin A than expanding process. This is explained in the following way: In fat-soluble vitamins, esters are significantly more stable than alcohols. The hydroxyl group of alcohols is extremely sensitive to oxidation. The five double bonds in retinol acetate still make the compound sensitive to oxidations. Since vitamin A is more stable in vitamin premixes than in vitamin-trace mineral premixes (trace minerals catalyze oxidation of the five double bonds) (Coelho, 2002). In pelleting, the most important adverse factors are friction (abrasion), pressure, heat, humidity and conditioning time. Friction and pressure expose more vitamin molecules to chemical destruction. Heat and humidity accelerate most chemical reactions. In extrusion, the dominant effects are pressure, heat, humidity and redox reactions. Extrusion is considered the most aggressive

process against vitamins due to the high temperatures (100-150°C), pressure (400-1000 PSI), and moisture (30%). Since humidity is significantly more stressful than temperature, in pelleting humidity, temperature and conditioning time is destroying vitamins at a higher rate than in the extrusion process. These findings are in accordance with the statements of *Gadient and Fenster* (1994) and *Coelho* (2002).

During storage for three months under extreme conditions of Climate chamber Binder (temperature 60°C and humidity 80%) average concentrations of vitamin A in untreated feed samples for broilers and piglets decreased to 60% and 70% of their initial values, respectively. In the samples of pelleted feed for broilers and expanded feed for piglets, the concentrations of vitamin A decreased to 39% and 50% of their initial values, respectively. After first month, the difference in vitamin A stability in untreated feed samples (UTB and UTP) was higher than in treated feed samples, however, it was not significant (P > 0.05), which is not case after three months of storage, when this distinction was significant (P < 0.05). This is important difference in terms of nutritional, but economic aspects also.

Vitamin A showed the adequate stability within the three month long period of storage, taking into account extreme storage conditions in a climate chamber.

According to the results of the study we can conclude that vitamin A could be stable if it is stored for a twelve months under normal, controlled conditions.

# CONCLUSION

Vitamin A from the examined untreated samples of feed for broilers and piglets was more stabile in comparison to the same nutrient in the pelleted and expanded samples. The content of vitamin A decreased to 60% and 70% of their initial values, respectively. In the samples of pelleted feed for broilers and expanded feed for piglets, the concentrations of vitamin A, after three months under extreme conditions in climate chamber, decreased to 39% and 50% of their initial values, respectively. Pelleting and expanding processes had significant effects on vitamin A stability in animal feeds, however the pelleting process has a greater impact on the degradation of vitamin A than expanding process. Furthermore, future studies should consider some other possible way of vitamin A application into animal feed, which will not have negative impact on vitamin A stability.

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