Original Research Article

Effects of starch and gelatin encapsulated vitamin A on growth performance, immune status and antioxidant capacity in weaned piglets

Yuhan Hu, Lin Zhang, Yu Zhang, Haitao Xiong, Fengqin Wang, Yizhen Wang, Zeqing Lu*

National Engineering Laboratory of Bio-feed Safety and Pollution Prevention; Key Laboratory of Animal Feed and Nutrition of Zhejiang Province; Key Laboratory of Animal Nutrition and Feed Science (Eastern of China), Ministry of Agriculture; Institute of Feed Science, Zhejiang University, Hangzhou 310058, China

A R T I C L E   I N F O

Article history:
Received 5 November 2019
Received in revised form 23 December 2019
Accepted 14 January 2020
Available online 24 February 2020

Keywords:
Starch encapsulated vitamin A
Gelatin encapsulated vitamin A
Antioxidant capacity
Growth performance
Immunity
Piglets

A B S T R A C T

To evaluate the effects of gelatin and starch encapsulated vitamin A on growth performance, immune status and antioxidant capacity in weaned piglets, a total of 96 weaned piglets (body weight = 9.11 ± 0.03 kg, 30-d-old) were randomly allotted to 3 treatments with 4 replications of 8 piglets each. The 3 treatments were control diet (basal diet without addition of vitamin A), gelatin vitamin A diet (basal diet + 13,500 IU/kg gelatin encapsulated vitamin A), and starch vitamin A diet (basal diet + 13,500 IU/kg starch encapsulated vitamin A), respectively. The results showed that piglets fed starch vitamin A diet had significantly higher final body weight and average daily gain compared to those in control and gelatin vitamin A groups (P < 0.05). Gelatin and starch vitamin A supplementation both highly increased serum retinol concentration and immunoglobulin (Ig) M level when compared with the control group (P < 0.05). Additionally, serum IgA level and glutathione peroxidase (GSH-Px) activity were significantly increased by gelatin vitamin A diet on d 21 and starch vitamin A diet on d 42, respectively (P < 0.05). These results demonstrated that dietary supplementation of vitamin A could improve immune function and antioxidant capacity in weaned piglets, and starch vitamin A is better than gelatin vitamin A, especially in promoting the growth performance of piglets.

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1. Introduction

Vitamin A plays important physiological roles in regulation of animal health, including the modulation of visual, reproductive, immune functions, and the growth and development of bone and tissue (Bondi and Sklan, 1984). As animals cannot synthesize vitamin A, its dietary supplementation is required to meet daily demands (Guilbert et al., 1940). However, plants contain only β-carotene (a precursor of vitamin A); natural vitamin A exists only in animal tissues (Thompson, 1975). Moreover, vitamin A extracted from plants or animal tissues are costly and difficult to procure in large quantity. Thus, vitamin A added in food or feed is primarily produced by commercial industrial synthesis (Maugard and Legoy, 2000). Indeed, the global demand of vitamin A reached approximately 24,700 t per year, and 85% of which was used in the feed industry (Marshall et al., 2008).

Vitamin A degrades easily due to its sensitivity to heat, light, moisture, and oxygen (Favaro et al., 2011). Thus, it must be protected by microencapsulation when added to the diet (Kim et al., 2000). Currently, vitamin A used for dietary purposes is mainly encapsulated by using gelatin (Donhowe and Kong, 2014), which is produced from animal bones, skin, and tendons. These animal sources are associated with the shortcomings of source instability, poor security, high cost, and excessive amounts of cadmium and other heavy metals (Schuurman et al., 2013). In recent years, a new form of encapsulated vitamin A via using starch has been...
developed, which shows better security and lower cost (Mun et al., 2015). However, there is no comparative data on the efficacy of different types of encapsulated vitamin A.

Herein, in this study, we compared the efficacy of these types of encapsulated vitamin A on growth performance, immune status, and antioxidant capacity in weaned piglets.

2. Materials and methods

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Zhejiang University (Hangzhou, China).

Gelatin and starch encapsulated vitamin A were provided by Zhejiang NU Co., Ltd (Xinchang, China). Vitamin A content of each formulation was 1.0 x 10^6 IU/g.

2.1. Animals, treatments, and housing

A total of 96 healthy Yorkshire × Landrace × Duroc weaned piglets (30-d-old) with an initial average body weight (9.11 ± 0.03 kg) were randomly allotted to 3 treatments with 4 replications of 8 piglets each pen (half male and half female). Piglets in the 3 dietary treatments were fed control diet (basal diet without addition of vitamin A), gelatin vitamin A diet (basal diet + 13,500 IU/kg gelatin encapsulated vitamin A), starch vitamin A diet (basal diet + 13,500 IU/kg starch encapsulated vitamin A), respectively. The whole trial period lasted for 42 d and was divided into 2 periods, d 0 to 21 and d 22 to 42. The basal diet was formulated to meet the (National Research Council, 2012) nutrient requirements. Composition and nutrient levels of the basal diet are presented in Table 1. Piglets were reared in identical environments and allowed ad libitum access to feed and water. The average room temperature and relative humidity were maintained at 24 to 26 °C and 60% to 70%, respectively.

2.2. Growth performance

Individual body weight of pigs was recorded at d 0 and 42 of the experimental period, and weighing was carried out after fasting. Feed consumption was recorded every 3 d. At the end of the experiment, average daily feed intake (ADFI), average daily gain (ADG), and the ratio of feed to gain (F:G ratio) were calculated based on these values.

2.3. Sample preparation and analysis

On d 21 and 42 of the experiment, 2 pigs (close to the average body weight) per pen were randomly selected from each group and venous blood was obtained by anterior vena cava puncture using 5-mL dipotassium ethylenediaminetetraacetic acid (EDTA-K2) tubes. Serum was obtained by centrifugation at 1,500 x g for 30 min, and samples were stored at −20 °C until analysis.

Serum retinol content was quantitatively analyzed using a Waters Alliance 2695 HPLC system equipped with a 2,998 PDA detector (Urbanek et al., 2006). The sample was loaded on a C18 column (250 mm x 4.6 mm, 2.5 μm, Waters, USA) and eluted with 100% methanol at 1.0 mL/min flow rate. The detection wavelength was 325 nm. The vitamin A standard (Retinol-synthetic, R7632) was purchased from Sigma (USA).

Serum immunoglobulins (IgG, IgM, and IgA) were determined using commercial enzyme linked immunosorbsent assay (ELISA) kits (Elabscience, Wuhan, China). Total antioxidant capacity (T-AOC), and lysozyme, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities were measured via colorimetric methods using a Microplate Reader (SpectraMax M5, Molecular Devices, USA). Assay kits for these tests were purchased from Nanjing Jian Cheng Bioengineering Institute (China).

2.4. Statistical analysis

Data were analyzed by one-way ANOVA of SPSS 16.0 (IBM-SPSS Inc., Chicago, USA) and compared using Tukey post-hoc test. P < 0.05 was considered to be statistically significant.

3. Results

3.1. Growth performance

Piglets fed starch vitamin A diet showed significantly higher final weight and ADG (P < 0.05), as well as a slightly reduced F:G ratio (P > 0.05) compared to control and gelatin vitamin A groups throughout the experimental period (Table 2). However, vitamin A supplementation had no effect on ADFI.

3.2. Serum retinol content

There was no significant difference in serum retinol content among the treatments on d 21 (Table 3). However, adding gelatin and starch vitamin A significantly increased serum retinol content by 43.10% and 60.95% when compared with the control group on d 42 (P < 0.05). Furthermore, pigs fed starch vitamin A diet tended to have a higher (15.76% in d 21 and 12.48% in d 42, P > 0.05) serum retinol content compared to gelatin vitamin A diet during the overall period.

3.3. Immunoglobulin and lysozyme

As shown in Table 4, on d 21, serum IgM level in vitamin A treatments was significantly higher than that in the control group (P < 0.05). Gelatin vitamin A supplementation significantly improved IgA level compared with the control group only (P < 0.05). On d 42, starch vitamin A supplementation significantly improved serum IgA level compared with the control group (P < 0.05). However, no significant difference in IgG level and lysozyme activity was observed among treatments during the overall period.
Previous studies have demonstrated that adding vitamin A to animal feed significantly improves ADG in weaned piglets and significantly decreases F:G ratio in growing-finishing pigs (Stahly et al., 1997). In the present study, on d 30 of the experiment, a part of piglets in the control group showed typical symptoms of vitamin A deficiency, including significant neurological symptoms, head and neck skewing, and difficulty in walking. These symptoms indicated that natural vitamin A provided by the feedstuff is far below the requirements. When used as additive, vitamin A is encapsulated to prevent oxidation. Suitable coating materials ensure the stability of vitamin A and improve absorption and utilization. Gelatin is generally used as a coating material, but in recent years, increasing attention has been paid to different materials, including contamination with excessive heavy metals and unstable supply levels (Zhang et al., 2013). In this study, a novel starch encapsulated vitamin A with improved security was used. Piglets receiving starch vitamin A had a significantly higher ADG than that receiving gelatin vitamin A. However, further stability tests and simulated gastrointestinal digestion trials in vitro are needed to explore these possibilities.

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Serum retinol content was used as an index to monitor the nutritional status of vitamin A (Naylor and Newcomer, 1999). On d 21, we found no significant effect of vitamin A addition on the serum retinol content. This may due to the storage of parent-derived vitamin A in the liver by piglets during the lactation period (Quadro et al., 2005). When a deficiency occurs, vitamin A stored in the liver is released to the blood to meet physiological needs.

### Table 2

| Item                  | Control  | Gelatin vitamin A | Starch vitamin A | SEM  | P-value |
|-----------------------|----------|-------------------|------------------|------|---------|
| Initial weight, kg    | 9.10     | 9.12              | 9.11             | 0.03 | 0.983   |
| Final weight, kg      | 33.04b   | 32.92b            | 34.38a           | 0.29 | 0.034   |
| ADFI, g/d             | 903      | 893               | 914              | 8.02 | 0.627   |
| ADG, g/d              | 570b     | 566b              | 802a             | 6.73 | 0.030   |
| F:G ratio             | 1.58     | 1.58              | 1.52             | 0.02 | 0.199   |

SEM = standard error of the mean (n = 4, number of replicates); ADFI = average daily feed intake; ADG = average daily gain; F:G ratio = ratio of feed to gain.

1. Control, basal diet without addition of vitamin A; Gelatin vitamin A, basal diet +13,500 IU/kg gelatin encapsulated vitamin A; Starch vitamin A, basal diet +13,500 IU/kg starch encapsulated vitamin A.

### Table 3

| Item                  | Control  | Gelatin vitamin A | Starch vitamin A | SEM  | P-value |
|-----------------------|----------|-------------------|------------------|------|---------|
| Day 21                | 67.93    | 64.14             | 74.25            | 2.07 | 0.125   |
| Day 42                | 46.20b   | 66.11a            | 74.36a           | 3.57 | 0.001   |

SEM = standard error of the mean (n = 8, number of replicates).

1. Control, basal diet without addition of vitamin A; Gelatin vitamin A, basal diet +13,500 IU/kg gelatin encapsulated vitamin A; Starch vitamin A, basal diet +13,500 IU/kg starch encapsulated vitamin A.

### Table 4

| Item                  | Control  | Gelatin vitamin A | Starch vitamin A | SEM  | P-value |
|-----------------------|----------|-------------------|------------------|------|---------|
| Day 21                | 35.36b   | 70.32a            | 48.61b           | 5.69 | 0.015   |
| Day 42                | 35.72b   | 57.35ab           | 81.51a           | 8.11 | 0.037   |

SEM = standard error of the mean (n = 8, number of replicates); IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M.

1. Control, basal diet without addition of vitamin A; Gelatin vitamin A, basal diet +13,500 IU/kg gelatin encapsulated vitamin A; Starch vitamin A, basal diet +13,500 IU/kg starch encapsulated vitamin A.
needs. Despite differences among the control and experimental groups in the levels of vitamin A consumed, serum retinol content was maintained at stable levels (approximately 65 μg/L), similar to that observed in previous studies (Anderson et al., 1995). However, at the end of the experiment, adding vitamin A to the diet significantly increased serum retinol content, indicating that vitamin A stored in the liver was gradually depleted in the control group. Over time, this would gradually affect the physiological function of the piglets in the control group. In contrast, the serum retinol contents in the experimental groups were relatively stable during the entire experimental period.

Lipid peroxidation occurs when oxygen free radicals react with cell membrane lipids, causing changes in membrane permeability and fluidity. This leads to metabolic disorders and immune dysfunction and ultimately causes serious injury to the animal nervous system or organs (Sohlenius-Sternbeck et al., 2000). The diene conjugate key in vitamin A molecules acts as an effective quencher and scavenger of lipid peroxidation free radicals, hydroxyl radicals, and other free radicals (Naylor and Newcomer, 1999). Total antioxidant capacity reflects the body's antioxidant defense system, including enzymatic and non-enzymatic systems. Our study showed that starch vitamin A slightly improved T-AOC in piglets during the whole experimental period. The enzymatic antioxidant system comprises SOD, GSH-Px, catalase, and other enzymes (Linnane et al., 2007). Indeed, in the current study, GSH-Px activity was significantly improved by adding gelatin or starch vitamin A. GSH-Px is a kind of peroxide decomposition enzyme with a selenocysteine active terminus of RBP. Biochemistry-US 1999;38(9):2647–2651. The authors declare there is no conflict of interest.

5. Conclusion

In conclusion, both gelatin and starch vitamin A can improve oxidation resistance and immune function in piglets. No significant difference was found between the 2 groups with regard to the physiological functions. However, in terms of the growth performance, piglets consuming starch vitamin A showed significantly higher final body weight and ADG than those in piglets consuming gelatin vitamin A. Our experimental results provide a strong rationale basis for the utilization of starch encapsulated vitamin A.

Acknowledgment

This study was supported by China Pig Modern Industrial Technology System (CARS-35).

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