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Original Research Article

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

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ABSTRACT

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.

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

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Table 1
Composition and nutrient levels of the basal diet (air-dry basis, %).

Ingredients	Content	Nutrient levels	Content
Corn	26.70	Metabolizable energy, MJ/kg	13.72
Expanded corn	15.00	Crude protein	20.00
Wheat flour	20.00	Calcium	0.83
Fermented soybean meal	8.00	Total phosphorus	0.55
Dehulled soybean meal	7.60	Lysine	1.20
Extruded soybean	7.00	Threonine	0.87
Fish meal	1.50	Vitamin A, IU/kg	667
Soy oil	2.00		
Dried porcine solubles	2.50		
Powdered milk	2.00		
Sucrose	1.50		
Monocalcium phosphate	1.10		
Limestone	1.10		
Premix[1]	4.00		

[1] Premix provided the following per kilogram diet: vitamin D_3, 3,000 IU; vitamin E, 100 mg; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 40 μg; biotin, 300 μg; folic acid, 2 mg; niacin, 45 mg; pantothenic acid, 20 mg; choline, 450 mg; vitamin C, 160 mg; Fe (ferrous sulfate), 150 mg; Cu (copper sulfate), 120 mg; Mn (manganese sulfate), 45 mg; Zn (zinc sulfate), 150 mg; I (calcium iodate), 0.3 mg; Co (cobalt nitrate), 0.3 mg; Se (sodium selenite), 0.3 mg.

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 NHU Co., Ltd (Xinchang, China). Vitamin A content of each formulation was 1.0×10^6 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 ($EDTAK_2$) tubes. Serum was obtained by centrifugation at 1,500 × *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 × 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 immunosorbent 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.

Table 2
Effects of vitamin A on growth performance of piglets.[1]

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	33.04[b]	32.92[b]	34.38[a]	0.29	0.034
ADFI, g/d	903	893	914	8.02	0.627
ADG, g/d	570[b]	566[b]	602[a]	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.
[a,b] Different superscripts within a row indicate significant differences ($P < 0.05$).
[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
Effects of vitamin A on serum retinol concentrations in piglets (μg/L).[1]

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	46.20[b]	66.11[a]	74.36[a]	3.57	0.001

SEM = standard error of the mean ($n = 8$, number of replicates).
[a,b] Different superscripts within a row indicate significant differences ($P < 0.05$).
[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.

3.4. Antioxidant properties

Dietary vitamin A supplementation had no significant effects on serum T-AOC and SOD on d 21 and 42 (Table 5). Whereas, serum GSH-Px activity was significantly increased by starch vitamin A diet on d 21 and gelatin vitamin A diet on d 42 ($P < 0.05$). There was no significant difference between starch and gelatin vitamin A on oxidation function in piglets.

4. Discussion

Vitamin A is an essential nutrient for growth and reproduction. 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 difficulties associated with procuring and using gelatin 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.

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

Table 5
Effects of vitamin A on serum antioxidant function in piglets (U/mL).[1]

Item	Control	Gelatin vitamin A	Starch vitamin A	SEM	*P*-value
Day 21					
T-AOC	1.02	1.28	1.72	0.18	0.376
SOD	73.47	70.35	73.48	1.75	0.476
GSH-Px	630.00[b]	663.52[ab]	700.69[a]	11.05	0.024
Day 42					
T-AOC	1.30	1.19	1.61	0.12	0.334
SOD	75.92	72.31	75.64	1.19	0.469
GSH-Px	789.51[b]	871.55[a]	828.25[ab]	13.40	0.012

SEM = standard error of the mean ($n = 8$, number of replicates); T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase.
[a,b] Different superscripts within a row indicate significant differences ($P < 0.05$).
[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
Effects of vitamin A on serum immunoglobulins in piglets.[1]

Item	Control	Gelatin vitamin A	Starch vitamin A	SEM	*P*-value
Day 21					
IgA, mg/L	35.36[b]	70.32[a]	48.61[b]	5.69	0.015
IgG, g/L	0.93	1.03	1.08	0.05	0.451
IgM, mg/L	88.33[b]	138.35[a]	133.56[a]	9.93	0.035
Lysozyme, U/mL	32.51	38.95	38.55	2.94	0.687
Day 42					
IgA, mg/L	35.72[b]	57.35[ab]	81.51[a]	8.11	0.037
IgG, g/L	0.90	1.07	1.04	0.04	0.111
IgM, mg/L	79.21	91.71	106.56	6.92	0.284
Lysozyme, U/mL	43.92	54.89	44.69	3.30	0.398

SEM = standard error of the mean ($n = 8$, number of replicates); IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M.
[a,b] Different superscripts within a row indicate significant differences ($P < 0.05$).
[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 center. Previous studies have revealed that vitamin A has interactions with zinc, iron, and copper, which indicated that vitamin A may improve GSH-Px activity through interactions with selenium (Rahman et al., 1995).

Vitamin A can alter the body's immune response. Vitamin A has effects on cell-mediated immunity by influencing the T helper cells (Thl and Th2) differentiation via retinoic acid receptor signaling (Cassani et al., 2012). Likewise, vitamin A can affect animal humoral immunity, which can lead to increased antibody generation (Surman et al., 2012). Here we found that adding vitamin A to the diet significantly increased serum IgA and IgM levels, which is consistent with the results of the previous studies (Michaelsson et al., 2003). Lysozyme, a hydrolytic enzyme specially made for microbial cell wall, which belongs to the non-specific immune system, has a bacteriolysis activity, can enhance immunity and accelerate tissue recovery functions (Callewaert and Michiels, 2010). In our study, throughout the whole experimental period, there was no significant difference in lysozyme content between control group and experimental groups.

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.

Conflict of Interest

The authors declare there is no conflict of interest.

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References

Anderson Sr LE, Myer RO, Brendemuhl JH, McDowell LR. The effect of excessive dietary vitamin A on performance and vitamin E status in swine fed diets varying in dietary vitamin E. J Anim Sci 1995;73(4):1093–8.
Bondi A, Sklan D. Vitamin A and carotene in animal nutrition. Prog Food Nutr Sci 1984;8(1–2):165–91.
Callewaert L, Michiels CW. Lysozymes in the animal kingdom. J Biosci 2010;35(1):127–60.
Cassani B, Villablanca EJ, De Calisto J, Wang S, Mora JR. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. Mol Aspect Med 2012;33(1):63–76. https://doi.org/10.1016/j.mam.2011.11.001.
Donhowe EG, Kong FB. Beta-carotene: digestion, microencapsulation, and in vitro bioavailability. Food Bioprocess Technol 2014;7(2):338–54. https://doi.org/10.1007/s11947-013-1244-z.
Favaro RMD, Iha MH, Mazzi TC, Favaro R, Bianchi MDP. Stability of vitamin A during storage of enteral feeding formulas. Food Chem 2011;126(3):827–30. https://doi.org/10.1016/j.foodchem.2010.09.014.
Guilbert HR, Howell CE, Hart GH. Minimum vitamin A and carotene requirements of mammalian species. J Nutr 1940;19(1):91–103.
Kim YS, Strand E, Dickmann R, Warthesen J. Degradation of vitamin A palmitate in corn flakes during storage. J Food Sci 2000;65(7):1216–9. https://doi.org/10.1111/j.1365-2621.2000.tb10268.x.
Linnane AW, Kios M, Vitetta L. Healthy aging: regulation of the metabolome by cellular redox modulation and prooxidant signaling systems: the essential roles of superoxide anion and hydrogen peroxide. Biogerontology 2007;8(5):445–67. https://doi.org/10.1007/s10522-007-9096-4.
Marshall RC, Marx LM, Raiff ME. Cartel price announcements: the vitamins industry. Int J Ind Organ 2008;26(3):762–802. https://doi.org/10.1016/j.ijindorg.2007.07.001.
Maugard T, Legoy MD. Enzymatic synthesis of derivatives of vitamin A in organic media. J Mol Catal B Enzym 2000;8(4–6):275–80. https://doi.org/10.1016/S1381-1177(99)00078-8.
Michaelsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. N Engl J Med 2003;348(4):287–94. https://doi.org/10.1056/NEJMoa021171.
Mun S, Kim YR, McClements DJ. Control of beta-carotene bioaccessibility using starch-based filled hydrogels. Food Chem 2015;173:454–61. https://doi.org/10.1016/j.foodchem.2014.10.053.
Naylor HM, Newcomer ME. The structure of human retinol-binding protein (RBP) with its carrier protein transthyretin reveals an interaction with the carboxy terminus of RBP. Biochemistry-Us 1999;38(9):2647–53. https://doi.org/10.1021/Bi982291i.
National Research Council. Nutrient requirements of swine, 11th revised edition. Washington, DC, USA: National Academies Press; 2012.
Quadro L, Hamberger L, Gottesman ME, Wang FW, Colantuoni V, Blaner WS, et al. Pathways of vitamin A delivery to the embryo: insights from a new tunable model of embryonic vitamin A deficiency. Endocrinology 2005;146(10):4479–90. https://doi.org/10.1210/en.2005-0158.
Rahman AS, Kimura M, Yokoi K, Tanvir EN, Itokawa Y. Iron, zinc, and copper levels in different tissues of clinically vitamin A-deficient rats. Biol Trace Elem Res 1995;49(1):75–84. https://doi.org/10.1007/BF02789004.
Schuurman W, Levett PA, Pot MW, van Weeren PR, Dhert WJA, Hutmacher DW, et al. Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. Macromol Biosci 2013;13(5):551–61. https://doi.org/10.1002/mabi.201200471.
Sohlenius-Sternbeck AK, Appelkvist EL, DePierre JW. Effects of vitamin A deficiency on selected xenobiotic-metabolizing enzymes and defenses against oxidative stress in mouse liver. Biochem Pharmacol 2000;59(4):377–83.
Stahly TSC, Cook DR, Ewan RC. Dietary vitamin A, E, C needs of pigs experiencing a low or high level of antigen exposure. Swine Res Rep 1997;75:194.
Surman SL, Rudraraju R, Sealy R, Jones B, Hurwitz JL. Vitamin A deficiency disrupts vaccine-induced antibody-forming cells and the balance of IgA/IgG isotypes in the upper and lower respiratory tract. Viral Immunol 2012;25(4):341–4. https://doi.org/10.1089/vim.2012.0023.
Thompson SY. Role of carotene and vitamin A in animal feeding. World Rev Nutr Diet 1975;21:224–80.
Urbanek L, Solichova D, Melichar B, Dvorak J, Svobodova I, Solich P. Optimization and validation of a high performance liquid chromatography method for the simultaneous determination of vitamins A and E in human serum using monolithic column and diode-array detection. Anal Chim Acta 2006;573–574:267–72. https://doi.org/10.1016/j.aca.2006.02.032.
Zhang NZ, Liu HS, Yu L, Liu XX, Zhang L, Chen L, et al. Developing gelatin-starch blends for use as capsule materials. Carbohydr Polym 2013;92(1):455–61. https://doi.org/10.1016/j.carbpol.2012.09.048.