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Araştırma Makalesi–Research Paper

LEVELS OF A AND E VITAMINS IN SOME VETERINARY PREPARATIONS KEPT IN DIFFERENT STORAGE CONDITIONS

FARKLI DEPOLAMA KOŞULLARINDA TUTULAN BAZI VETERİNER HEKİMLİĞİ PREPARATLARINDA A VE E VİTAMİNLERİNİN DÜZEYLERİ

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Özet

Bu çalışma, Veteriner Hekimler tarafından yaygın şekilde kullanılan, farklı koşullarda saklanan A ve E vitaminlerinin benzer kombinasyonlarına sahip olan preparatlarda vitamin A ve E düzeylerinin değişikliklerini araştırmayı amaçladı. Kapağı açılan ve açılmayan preparatlar 25 °C'de karanlık ve aydınlıkta ve 4 °C'de karanlıkta tutuldu. Vitamin A ve E düzeyleri 0, 7, 14, 28 ve 56. günlerde analiz edildi. Mevcut US Farmakope'si tarafından modifiye ve doğrulanmış bir metot vitaminlerin analizleri için kullanıldı. Kapağı açılan ve açılmayan şişelerde A ve E vitaminlerinin zamana göre değişme oranları üzerine 25 °C'de karanlık ve aydınlığın ve 4 °C'de karanlığın etkisinin vitamin E için yüksek olduğu ve vitamin A için düşük olduğu bulundu. Ayrıca kapağı açılan preparatlarda vitamin A ve E'nin zamana göre değişme oranları 25 °C'de tutmaya göre 4 °C'de daha yüksekti. Kapağı açılmayan preparatlarda vitamin A ve E düzeyleri büyük ölçüde korundu. Farklı firmalardan temin edilen preparatların ilk kullanımını takiben ışık maruziyeti olmadan ve oda sıcaklığında şişeleri kutularda tutmanın uygun depolama koşulları olacağı sonucuna varıldı.

Anahtar Kelimeler: Depolama koşulları, Stabilite, Vitamin A, Vitamin E

Abstract

This study aimed to investigate the changes of the vitamins A and E levels in preparations with similar combinations of vitamins A and E, stored in different conditions, widely used by veterinarians. The unsealed and sealed preparations were kept in dark and light at 25 °C and in dark at 4 °C. The levels of vitamins A and E were analyzed on days 0, 7, 14, 28 and 56. A modified and validated method by the current US Pharmacopeia was used for the analyses of vitamins. In the unsealed and sealed bottles, the effect of light and dark at 25 °C, and of dark at 4 °C to time on the changing rates of A and E vitamins was found to be higher for vitamin E, and to be lower for vitamin A. Moreover, the changing rates of vitamins A and E with respect to time for the unsealed preparations were higher at 4 °C than keeping at 25 °C. The levels of vitamins A and E were substantially protected in the sealed preparations. It was concluded that keeping bottles in their boxes in room temperature and without exposure of light following the first use of preparations supplied from different firms would be suitable storage conditions.

Keywords: Storage conditions, Stability, Vitamin A, Vitamin E

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1. INTRODUCTION

A small amount of dietary vitamin intake is required for maintaining normal metabolic functions in cells (Blumberg et al., 2018, pp. 640-657). Vitamins have functions as adjunct factor for various enzymes in protein, glucose and fat metabolism (Borges et al., 2018, pp. 106-115; He et al., 2019, pp. 3182-3191; Nakaoka et al., 2018, pp. 23-31). Vitamin deficiencies cause various diseases, growth retardation, and reproductive failure (Divers et al., 2006, pp. 120-126; Hurley and Doane, 1989, pp. 784-804; Li et al., 2019, pp. 3354-3365; McGill et al., 2019, pp. 15157). Although animals meet vitamin needs via feeds, vitamin supplementations may be sometimes required for them during pregnancy, lactation, and egg production due to work load (Santos et al., 2019, pp. 4694-4704; Wang et al., 2018, pp. 2098-2104). In addition, various vitamin formulations are used in prevention and treatment of diseases (Jiang, 2014, pp. 76-90, Zinder at al., 2019, pp. 839-849).

The activity and stability of vitamins are affected by various factors such as air, oxygen, temperature, light, and chemical substances (Kondepudi, 2016, pp. 499-503; Kuong et al., 2016, pp. 51). The stability of active ingredient is an important point for drug design and development. The properties of the product should be protected during production, storage, transport, and usage. In addition, company is responsible for the stability of the product (Acartürk, 2009, 141-182). The stability changes of vitamins have been reported in extruded fish feeds (Anderson and Sunderland, 2002, pp. 137-149; Gadient and Fenster, 1994, pp. 207-211) and in broiler premix diet due to added minerals (Zhuge and Klopfenstein, 1986, pp. 987-994). Thus, vitamin stabilities in formulations are important point for the effective treatment of diseases, the effective nutrition and maintenance of good health in animal or human. Veterinarians prefer to use vitamin formulations sold in large packages due to economic concerns. Following initial use of preparation, remaining drug in bottle is used in different times. When drug bottles are not stored under suitable conditions, their efficacies and stabilities can be changed. In addition, the different information in prospectus of vitamin preparations causes problems on the suitable storage conditions. Therefore, this study aimed to determine the most suitable storage conditions among suggestions of storage conditions in prospectuses of preparations by statistically evaluating the changes of vitamins A and E in different storage conditions of similar preparations containing A and E vitamins, widely used by veterinarians.

2. MATERIALS AND METHODS

2.1. Chemical substances

Methanol (Merck Lot No: I748007 433), distilled water (Lot No: Z0331233 431 Merck), Vitamin A (99.0%, 0.15 g, Lot No: 20607) and Vitamin E (98.5%, 0.5 g, Lot No: 20502) were obtained.

2.2. Laboratory wares

Volumetric flask (50 ml-100 ml), beaker (100 ml), injector 20 ml, pipette tips and

routine laboratory wares were used.

2.3. Pharmaceutical preparations

The preparations were obtained from three different firms and coded as A, B, and C (Ademin, Ceva Dif, İstanbul, Turkey; Adesol, Topkim, İstanbul, Turkey; Adevilin, Vilsan, Ankara, Turkey) respectively. These preparations contained 500.000 IU vitamin A and 50 mg vitamin E per 1 mL. In this study, the vitamins A and E were analyzed. After all preparations were supplied, the preparations were divided into two groups. After the preparations in first group were unsealed on day 0, they were analyzed for A and E vitamins on days 0, 7, 14, 28 and 56 with sampling from bottles kept in boxes (covered) in cupboard in the dark conditions at 25 °C and bottles (uncovered) in the light conditions at 25 °C. Bottles were kept in boxes (covered) in the dark conditions at 4 °C.

The preparations in second group were kept with seal in different time points at different conditions (covered or uncovered) and seal of bottle was opened only during the analyses, and bottles were discarded after each sampling for the analysis. Bottles were kept in boxes (covered) in cupboard in the dark conditions at 25 °C, and bottles were uncovered in the light conditions at 25 °C. Bottles were kept in boxes (covered) in the dark conditions at 4 °C.

2.4. Analyses of vitamins A and E

The levels of vitamins A and E were measured on days 0, 7, 14, 28 and 56, in dark and light room at 25 °C, and in dark at 4 °C. Vitamin A and vitamin E analyses were performed according to a modified and validated analytic method (ICH Topic Q2 (R1), validation of analytic procedures: text and methodology) via methods of US Pharmacopeia (USP, 2009, pp. 152) using a high pressure liquid chromatography (HPLC) (Serial No: L20225116387). Chromatographic conditions were as follows: Thermo scientific ODS hypersil C18 (Serial no: 10145776; Column properties: 250 mm × 4.6 mm × 5 µm); quaternary gradient pump, auto-sampler (SIL-20A ht), degasser (DGU-20As), UV detector or diode array detector (DAD) (SPD-20A), column oven (CTO-10AS vp), ultrasonic bath (serial no: 1471519), automatic pipette (Vitlap 1000 µL; serial no: 11L09323).

Chromatographic conditions included column thermo scientific, particle size of 5 µm, length of 250 mm, diameter of 4.6 mm, column temperature of 25 °C, flow rate of 1.0 mL/min, wavelength of 325 nm vitamin A, 280 nm vitamin E, injection volume of 20.0 µL, analysis time of 15 min, and mobile phase of methanol.

2.5. Statistical analysis

Statistical analyses for the levels of A and E vitamins were performed according to statistical package program SPSS (Ver. 20). Different temperature and environment conditions to time for vitamins were assessed using generalized linear models. Differences between means in independent group were evaluated using Bonferroni test. Differences of means of data in analyses of samples obtained from unsealed and sealed bottles to time were analyzed using variance analysis of repetitive measurements in independent groups. Data were showed

as means ± standard deviation. P values smaller than 0.05 was considered significant.

3. RESULTS

The levels of vitamin A in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 14, 28 and 56 days compared to on 0 day in light (uncovered and exposed to daylight) and dark (covered, in cupboard) conditions at 25 °C ($P<0.05$). The levels of vitamin A in the unsealed preparations of A and B firms were significantly decreased on day 7 ($P<0.01$) and on day 28 ($P<0.05$) in light conditions than in the dark conditions at 25 °C, respectively (Table 1). The vitamin A changes in the unsealed preparations of A, B, and C firms were 7.23, 4.65, 3.58% in the dark conditions, and 6.82, 5.4, 6.13% in the light conditions at 25 °C, respectively.

Table-1: Changes in Vitamin A Levels (IU) of Unsealed Preparations Kept in Light and Dark Conditions at 25 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Temperature**	**Light or Dark**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	25 °C	Light	535247,37± 14114,08^{a}	506895,94± 5871,85^{Bb}	510974,18± 10641,72^{bc}	521925,41± 10651,99^{db}	498738,66± 13618,43^{ec}
		Dark	544455,76± 9363,06^{a}	526269,32± 9210,65^{b}	507980,71± 11177,58^{c}	531997,04± 11736,96^{db}	505061,23± 18337,51^{ec}
(B)	25 °C	Light	521640,36± 8514,14^{a}	507225,94± 15497,97^{b}	496272,79± 12896,62^{c}	507750,80± 12509,07^{bd}	493047,88± 13677,71^{e}
		Dark	520844,00± 10797,48^{a}	508533,72± 11166,95^{b}	502969,26± 6667,83^{bc}	523900,26± 13539,19	496620,39± 24243,97^{e}
(C)	25 °C	Light	496991,62± 12525,14^{a}	486396,98± 10997,29^{b}	475123,25± 5780,78^{c}	480640,26± 14842,69^{dc}	466483,23± 17106,79^{e}
		Dark	496194,74± 9345,90^{a}	491469,27± 12087,42^{b}	480121,18± 11101,02^{c}	482466,42± 13237,10^{d}	478399,45± 14901,30^{e}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin A active ingredient to time in the same line ($p<0.05$). The letters of A and B show statistical significance for vitamin A changes in the same time and in different storage conditions, $p<0.05$ and $p<0.01$, respectively.

The levels of vitamin E in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 28 and 56 days compared to on 0 day in the light and dark conditions at 25 °C (Table 2). The vitamin E changes in the unsealed preparations of A, B, and C firms were 10.67, 12.76, 8.72% in dark conditions, and 10.47, 13.66, 6.7% in the light conditions at 25 °C, respectively.

Table-2: Changes in Vitamin E Levels (mg) of Unsealed Preparations Kept in Light and Dark Conditions at 25 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Temperature**	**Light or Dark**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	25 °C	Light	51,57±1,99^{a}	50,84±2,15^{b}	48,47±1,20^{bc}	46,17±0,76^{cd}	47,38±1,35^{ec}
		Dark	52,02±2,33^{a}	50,42±2,78^{b}	49,05±1,63^{c}	46,47±1,46^{d}	47,74±1,10^{ec}
(B)	25 °C	Light	57,49±0,82^{a}	55,91±2,26^{b}	54,34±1,54^{bc}	52,01±0,80^{d}	49,64±1,20^{e}
		Dark	58,94±1,50^{a}	58,09±2,95^{ab}	52,87±0,76^{c}	56,29±4,13^{db}	51,42±0,42^{ec}
(C)	25 °C	Light	48,86±1,70^{a}	47,68±1,77^{b}	45,82±1,36^{c}	45,61±1,64^{c}	45,59±1,93^{ec}
		Dark	50,01±1,02^{a}	48,81±1,49^{b}	46,30±0,69^{c}	45,65±0,70^{cd}	46,37±2,21^{ec}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin E active ingredient to time in the same line ($p<0.05$).

The levels of vitamin A in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 14, 28 and 56 days compared to on 0 day in the dark conditions at 25 °C and 4 °C. The levels of vitamin A in the unsealed preparations were significantly decreased on day 28 for A and B firms at 4 °C than 25 °C in the dark conditions (Table 3). The vitamin A changes in the unsealed preparations of A, B, and C firms were 7.23, 4.65, and 3.58% in the dark conditions at 25 °C and 7.58, 5.70, and 6.60% in the dark conditions at 4 °C, respectively.

Table-3: Changes in Vitamin A Levels (IU) of Unsealed Preparations Kept in Dark Conditions at 25 °C and 4 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Condition**	**Temperature**	**x ±sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	Dark	25 °C	544455,76 ±9363,06^{a}	526269,32 ±9210,66^{b}	507980,71 ±11177,58^{c}	531997,04 ±11736,96^{Acd}	505061,23 ±18337,51^{e}
		4 °C	543841,46 ±12280,83^{a}	525361,71 ±16783,83^{b}	512717,96 ±16452,69^{bc}	518044,06 ±13132,56^{db}	502595,94 ±14468,30^{e}
(B)	Dark	25 °C	520844,00 ±10797,48^{a}	508533,72 ±11166,95^{b}	502969,26 ±6667,83^{bc}	523900,26 ±13539,19^{Abc}	496620,39 ±24243,97^{e}
		4 °C	516823,30 ±14137,07^{a}	505805,47 ±19504,84^{b}	504246,89 ±8697,37^{c}	508597,36 ±11136,57^{bd}	487317,06 ±25212,36^{e}
(C)	Dark	25 °C	496194,74 ±9345,90^{a}	491469,27 ±12087,42^{b}	480121,18 ±11101,02^{A}	482466,42 ±13237,10^{d}	478399,44 ±14901,30^{e}
		4 °C	499658,71 ±13065,32^{a}	495661,55 ±11949,08^{b}	490388,33 ±5451,04^{c}	482698,68 ±17434,40^{cd}	466679,96 ±16909,36^{e}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin A active ingredient to time in the same line ($p<0.05$). The letter of A shows statistical significance for vitamin A changes in the same time and in different storage conditions ($p<0.05$).

The levels of vitamin E in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 14, 28 and 56 days compared to on 0 day in the dark conditions at 25 °C and 4 °C (Table 4). The levels of vitamin E in the unsealed preparations of B firm were significantly decreased on day 28 at 4 °C than at 25 °C and in the dark conditions. The vitamin E changes in the preparations of A, B, and C firms were 10.66, 12.75, 8.71% in the dark conditions at 25 °C and 11.49, 16.03, 9.03% in the dark conditions at 4 °C, respectively.

Table-4: Changes in Vitamin E Levels (mg) of Unsealed Preparations Kept in Dark Conditions at 25 °C and 4 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Condition**	**Temperature**	**x ±sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	Dark	25 °C	52,02±2,33^{a}	50,42±2,78^{b}	49,05±1,63^{bc}	46,47±1,47^{d}	47,74±1,10^{ed}
		4 °C	52,30±1,59^{a}	51,74±1,94^{b}	48,15±2,18^{c}	46,29±0,85^{d}	47,84±1,19^{ed}

(B)	Dark	25 °C	58,94±1,50^{a}	58,09±2,95^{ab}	52,87±0,76^{c}	56,29±4,13^{Abd}	51,42±0,42^{ed}
		4 °C	58,98±2,67^{a}	58,17±3,60^{ab}	54,83±0,84^{Ac}	52,33±0,99^{cd}	49,52±2,15^{e}
(C)	Dark	25 °C	50,01±1,02^{a}	48,81±1,49^{b}	46,30±0,69^{c}	45,65±0,71^{cd}	46,37±2,21^{ec}
		4 °C	50,36±0,59^{a}	49,76±0,41^{ab}	47,83±0,37^{c}	45,81±0,33^{d}	46,64±0,29^{ed}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin E active ingredient to time in the same line ($p<0.05$). The letter of A shows statistical significance for vitamin E changes in the same time and in different storage conditions ($p<0.05$).

The levels of vitamin A in the sealed preparations of A firm were not significantly different on day 56 compared to day 0 in the dark (covered, in cupboard) and light conditions (uncovered and exposed to daylight) at 25 °C, as well as in the dark condition for C firm, but significantly different for B firm in the dark and light conditions (Table 5). The vitamin A changes in the preparations of A, B, and C firms were 0.92, 0.54, 1.41% in the dark conditions, and 0.98, 0.73, 1.15% in the light conditions at 25 °C, respectively.

Table-5: Changes in Vitamin A levels (IU) of Sealed Preparations Kept in Light and Dark Conditions at 25 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Temperature**	**Condition**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	25 °C	Light	513172,85± 10080,70^{Ba}	508845,50± 9835,33^{b}	508124,06± 6747,92^{b}	510118,63± 8826,41^{ab}	513102,32± 7145,70^{a}
		Dark	510796,19± 7411,44^{a}	507804,24± 3843,06^{b}	505993,45± 5491,39^{c}	506081,88± 5647,28^{Bbd}	510373,56± 6390,53^{a}
(B)	25 °C	Light	505159,20± 9427,07^{a}	509702,33± 11926,70^{Bb}	501462,55± 7502,87^{Ac}	507298,9±6 547,52 ^{Bbd}	507561,83± 6883,01^{d}
		Dark	504924,00± 5276,79^{a}	502199,34± 6545,53^{bc}	503974,66± 6359,67^{bc}	504163,45± 5374,37^{a}	502959,77± 5049,46^{cd}
(C)	25 °C	Light	500034,034 ±3290,11^{a}	495665,39± 5291,00^{b}	498742,86± 7423,05^{c}	494261,02± 6362,17^{Ab}	503063,13± 9115,72^{d}

		Dark	502839,30± 7936,05^{a}	500296,03± 9059,88^{b}	495945,97± 8534,22^{Ac}	495752,73± 4831,79^{c}	502840,22± 6616,52^{a}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin A active ingredient to time in the same line ($p<0.05$). The letters of A and B show statistical significance for vitamin A changes in the same time and in different storage conditions, $p<0.05$ and $p<0.01$, respectively.

The levels of vitamin E in the sealed preparations of A firm were not significantly different between day 56 and day 0 in the dark conditions at 25 °C. The levels of vitamin E in the sealed preparations of B and C firms were significantly decreased on day 56 compared to day 0 in the light and dark conditions at 25 °C. The levels of vitamin E in the sealed preparations of A, B and C firms were significantly different between the dark and the light conditions at 25 °C (Table 6). The vitamin E changes in the preparations of A, B, and C firms were 0.43, 0.75, 0.54% in the dark conditions, and 0.26, 1.39, 0.58% in the light conditions at 25 °C, respectively.

Table-6: Changes in Vitamin E Levels (mg) of Sealed Preparations Kept in Light and Dark Conditions at 25 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Temperature**	**Condition**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	25°C	Light	49,38±0,95^{Aa}	49,69±0,85^{Ab}	49,588±0,43^{Ac}	49,25±0,50^{Aad}	49,63±1,14^{Ab}
		Dark	51,30 ±2,20^{a}	51,406±2,17^{b}	51,80±2,02^{c}	51,486±2,36^{bd}	51,08±2,10^{a}
(B)	25°C	Light	58,07±0,92^{a}	58,62±0,91^{b}	58,01±1,11^{a}	57,26±0,81^{c}	57,28±1,02^{c}
		Dark	50,71±0,88^{a}	50,58±1,36^{b}	50,801 ±0,82^{c}	50,38±0,64^{d}	50,33±1,00^{d}
(C)	25°C	Light	50,34±1,55^{Aa}	50,54±1,48^{Ab}	50,78±1,13^{Ac}	50,16±1,65 ^{Ad}	50,05±1,46^{e}
		Dark	52,18 ±0,86^{a}	52,14±0,77^{b}	52,12±0,72^{ab}	52,02±0,58^{c}	51,9±0,82^{d}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin E active ingredient to time in the same line ($p<0.05$). The letter of A shows statistical significance for vitamin E changes in the same time and in different storage conditions ($p<0.05$).

The levels of vitamin A in the sealed preparations of A firm were not significantly different between day 56 and day 0 in the dark condition at 25 °C but there was significant difference between day 56 and day 0 at 4 °C. The levels of vitamin A in the sealed preparations

of B and C firms were significantly different between day 56 and day 0 in the dark condition at 25 °C and 4 °C (Table 7). Vitamin A changes in the preparations of A, B, and C firms were 0.94, 0.54, 1.41% in the dark conditions at 25 °C, and 0.56, 0.82, 0.7% in the dark conditions at 4 °C.

Table-7: Changes in Vitamin A Levels (IU) of Sealed Preparations Kept in Dark Conditions at 25 °C and 4 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Condition**	**Temperature**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	Dark	25°C	510796,19± 7411,44^{a}	507804,24± 3843,06^{b}	505993,45± 5491,39^{bc}	506081,88± 5647,28^{Bcd}	510373,56± 6390,53^{ab}
		4 °C	511497,27± 66981,96^{a}	508640,83± 10239,34^{b}	510235,33± 7734,12^{ac}	511030,57± 6654,05^{ac}	509321,9±5 488,94^{b}
(B)	Dark	25°C	504924,00± 5276,79^{a}	502199,34± 6545,53^{bc}	503974,66± 6359,67^{bc}	504163,45± 5374,37^{a}	502959,77± 5049,46^{cd}
		4 °C	503653,03± 7939,07^{a}	499515,57± 11303,99^{Ab}	503185,43± 5957,52^{ac}	502530,57± 8267,75^{Ac}	501918,72± 6836,18^{d}
(C)	Dark	25°C	502839,30± 7936,05^{a}	500296,03± 9059,88^{b}	495945,97± 8534,22^{bc}	495752,73± 4831,79^{bc}	502840,22± 6616,52^{b}
		4 °C	499008,57± 3269,73^{a}	495501,1±7 507,52^{b}	502130,1±6 941,95^{bc}	497935,93± 5656,62^{cd}	498134,14± 6347,52^{d}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin A active ingredient to time in the same line ($p<0.05$). The letters of A and B show statistical significance for vitamin A changes in the same time and in different storage conditions, $p<0.05$ and $p<0.01$, respectively.

The levels of vitamin E in the sealed preparations of A firm were significantly decreased on day 56 and on 0 day in the dark condition at 25 °C but there was no significant difference between day 56 and day 0 in the dark condition at 4 °C. The levels of vitamin E in the preparations of B firm were not significantly different between day 56 and day 0 in the dark condition at 25 °C but there was a significant difference between day 56 and day 0 in the dark condition at 4 °C. The levels of vitamin E in the preparations of C firm were significantly decreased on day 56 compared to on 0 day in the dark condition at 25 °C but there was no significant difference between day 56 and day 0 at 4 °C (Table 8). The changes of vitamin E in the sealed preparations of A, B, and C firms were 0.43, 0.75, 0.54% in the dark conditions at 25 °C, and 1.63, 1.49, 0.08% in the dark conditions at 4 °C, respectively.

Table-8: Changes in Vitamin E Levels of Sealed Preparations Kept in Dark Conditions at 25 °C and 4 °C to Time

			Day 0	**Day 7**	**Day 14**	**Day 28**	**Day 56**
Firm	**Condition**	**Temperature**	**x ±sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
(A)	Dark	25 °C	51,30±2,20^{a}	51,406±2,17^{ab}	51,80±2,02^{c}	51,486±2,36^{b}	51,08±2,10^{d}
		4 °C	50,78±0,45^{a}	51,10±0,71^{b}	50,888±0,70^{ac}	49,95±0,36^{Aacd}	50,65±0,76^{ac}
(B)	Dark	25 °C	50,71±0,88^{a}	50,58±1,36^{a}	50,801±0,82^{a}	50,38±0,64^{b}	50,33±1,00^{a}
		4 °C	58,52±2,37^{a}	59,12±2,41^{b}	58,32±2,06^{ab}	58,30±1,64^{b}	57,65±2,12^{c}
(C)	Dark	25 °C	52,183±0,86^{a}	52,14±0,77^{b}	52,12±0,72^{b}	52,02±0,58^{bc}	51,90±0,82^{d}
		4 °C	48,74±1,92^{Ba}	49,02±1,75^{Bb}	49,053±1,63^{Bb}	48,70±1,84^{Ba}	48,77±1,46^{Aa}

The letters of a, b, c, d, e show statistical significance for the changes of vitamin E active ingredient to time in the same line ($p<0.05$). The letters of A and B show statistical significance for vitamin E changes in the same time and in different storage conditions, $p<0.05$ and $p<0.01$, respectively.

4. DISCUSSION

In this study, the changes of the levels of vitamins A and E were investigated in preparations comprising vitamins A and E frequently used in veterinary medicine and exposed to different storage conditions. According to the author's knowledge, there were no similar studies, but there were few studies about vitamin stabilities in feeds (Dove and Evan, 1991, pp. 1994-2000; Gadient and Fenster, 1994, pp. 207-211).

There is a number of environmental, physical and chemical factors to affect the stability of compounds and thus, these factors affect shelf life of preparations comprising vitamins. The most important factors are mainly temperature, humidity, oxygen, light, pH of product, oxidation and reduction substances and metal ions (iron and copper) (IADSA, 2014, pp. 32).

Stability of active ingredients in vitamin products can change in time and during the product shelf life (Berry Ottaway, 2008, pp. 88-107). A study has determined the losses of vitamin A (44%), vitamin C (23%), vitamin B12 (7.7%), and folic acid (10.5%) in multi-vitamin tablet (Berry Ottaway, 2008, pp. 88-107). In this study, the changes of the vitamins A and E levels of different vitamins in glass bottles were determined to be different in the result of the effects of temperature and light to time.

It has been stated that storage conditions of medicines are important and the reactions of

drugs with heat, humidity, light, etc. cause discoloration and efficacy changes in preparations and adverse effects on patient's health (Arshad et al., 2011, pp. 543-547)

It has been revealed that medicines are exposed to high temperatures in general practice during summer months and their efficacies would be adversely affected (Crichton, 2004, pp. 328-329) This study confirmed that temperature conditions had effects on the active ingredients in the preparations comprising vitamins. In this study, the highest percentages of the changes were determined in vitamin E compared to vitamin A at different temperatures with respect to time in unsealed preparations. In addition, decreases in the levels of vitamin A were higher at 4 °C than 25 °C to time in unsealed preparations. Thus, the changes in the levels of vitamins A and E were found to be higher at 4 °C than 25 °C in the unsealed vitamin preparations.

It has been found that the stability of vitamin E in premixes with choline chloride is not significantly decreased in dark condition at 25 °C and at 60% humidity during 3 months (Tavcar-Kalcher and Vengust**,** 2007, pp. 148-154). However, in this study, vitamin E stability in the preparations significantly changed on day 7 in the dark condition and at 25 °C.

Vitamins A and E are sensitive to oxidation. It is required to prevent formulations comprising these vitamins against oxygen, metal ions, and ultraviolet light (Combs, 1992, pp. 57-59; Kondepudi, 2016, pp. 499-503). In this study, it was found that the stabilities of vitamins were affected at different rates in the same storage conditions and that A and E vitamins were sensitive to various environmental conditions.

The storage of vitamin A is recommended in dark conditions (Combs, 1992, pp. 57-59). In this study, the rates of vitamin A in dark and light conditions less changed than E vitamin. It was also determined that vitamin A was suitable to be stored in dark conditions. E vitamin is recommended to be stored in cold conditions (Combs, 1992, pp. 57-59). However, in this study, the rates of vitamin E changes than vitamin A changes were found to be highest at different temperatures to time. In addition, the rates of vitamin E changes were found to be higher at 4 °C than at 25 °C.

A study has recommended the storage of tocopherols under cold, dark, and dry conditions (Wade and Weller, 1994, pp. 13-14). However, in this study, the storage of vitamin E was found to be suitable under room temperature and dark conditions. Thus, in this study, it was determined that such vitamins would be suitable to be stored under room temperature and dark conditions.

In this study, the changes of vitamins A and E with respect to time were less in the sealed preparations than the unsealed preparations in different temperature and dark and light conditions. Thus, active ingredients were considerably protected in sealed preparations based on vitamin changing rates.

5. COCLUSION

It was concluded that following the first use in the preparations supplied from different firms and kept in different conditions, keeping bottles in their boxes at room temperature and without exposure of light would be suitable storage conditions.

Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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