

Supplementation with a Combination of Vitamin A, E, C and Essential Volatile Oils Improves Growth Performance and Ameliorates Antioxidant Status in Holstein Calves

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Abstract

The aim of the present study was to evaluate the effect of supplementation with a combination of vitamin E, A and C and essential volatile oils (Super Immune®) on growth performance and antioxidant parameters in calves. For this purpose, twenty-five Holstein calves were randomly allocated into five equal sized groups (five each). The first group received a daily dose of 10 ml of Super Immune® which was given orally. The second group was supplemented with 100,000 IU Vit A and given intramuscularly. The third group was supplemented with Vit. E, at a dose rate of 6.66 IU/kg BW and given subcutaneously. Meanwhile, the fourth group was supplemented with Vit. C at a dose rate of 30 mg/kg and given intravenously. The former formula was given orally for five consecutive days, while the later supplements were given twice with 72 hours apart. Calves of the fifth group received a sterile saline solution (0.9 % NaCl) and served as control group. Calves received a combination of vitamins and volatile oils showed significant increase of the body weight compared with control group at 6 weeks post-treatment ($p < 0.05$). There was also a significant increase of total antioxidant capacity (TAC) ($p < 0.01$), Glutathione reductase (GR) ($p < 0.05$) and Glutathione-S-transferase (GST) ($p < 0.05$) in calves received combination compared with other groups. However, there was a significant decrease of malondialdehyde (MDA) ($p < 0.01$). On the other hand, other parameters showed non-significant changes. The results of the present study indicate that a combination of vitamin A, E and C and volatile oils (Super Immune®) may have better effect on body weight gain and antioxidant status in suckling calves than supplementation with single vitamin.

KEYWORDS

Calves, Vitamins, Volatile oils, Oxidative status, Oxidative stress

INTRODUCTION

In 2006, the European Union prohibited all antibiotics that can be used as growth promoters for animals and proposed potential alternatives (Simitzis *et al.*, 2017). Natural products, including medicinal plants, essential oils (EOs), and herbal extracts, can be promising alternatives (Mushtaq *et al.*, 2018). EOs are considered the most economically relevant plant-derived products and could likely have health-promoting properties (Nehme *et al.*, 2021). A study the effect of essential oil mixture (Eucalyptus oil, menthol and Mint oil) supplementation at different doses in milk replacer during pre-weaning period and in water during post-weaning period of the calves has been found to increase daily body gain, reduced feed intake and improved FCR and the higher dose of EOM administration less effective and uneconomical to be used (Soltan, 2009).

Vitamin E is considered the most potent antioxidant, responsible for the protection of membrane polyunsaturated fatty acid against lipid peroxidation. Because α -tocopherol does not cross the placenta, the calf is dependent on colostral intake to obtain vitamin E after birth but vitamin E content of colostrum is usually low unless the cow is provided with supplementary dietary vitamin E and so the best method for increasing the vitamin E content of colostrum is supplementing the newborn calves around calving with vitamin E (Mokhber-Dezfouli *et al.*, 2008). Vitamin E is necessary to protect the newborn against oxidative stress whereas vitamin A is required for growth and development; and both vitamins are essential for immune system development (Nayyar and Jindal, 2010). Administration of vitamin A (alone or in combination with iron) results in higher amounts of RBC parameters and weight gain than control calves (Moosavian *et al.*, 2010).

Vitamin C, Ascorbic acid is an antioxidant essential for nu-

merous cellular functions it acts as free radical scavenger. Dairy calves apparently do not produce endogenous vitamin C until 4 months of age and depend on low concentrations of vitamin C in milk and so it needs for ascorbic acid supplements rearing period (Seifi *et al.*, 2010).

Little is known about the effect of combination of vitamins and volatile oils on oxidant/antioxidant balance in calves. Therefore, the aim of the present study was to evaluate the Effect of supplementation with combination of vitamin E, A and C and volatile oils (Super Immune®) on growth performance and antioxidant parameters in calves. We hypothesize that a combination of EOM and vitamins could improve weight gain and ameliorate redox status in young calves.

MATERIALS AND METHODS

Animals

Twenty-five apparently healthy Holstein calves weighing 35-45 kg body weight at two weeks of age were used. Calves were raised at a commercial dairy farm and located at Dakahlia governorate, Egypt. The calves were investigated clinically, and the findings were recorded simultaneously. All animals were clinically healthy, with no history of ailments and were kept under identical housing and veterinary supervision throughout the study. All applicable international guidelines for animal care and the use of animals for scientific purposes were also followed. The present study has been approved by the Animal Care and Use Committee, Mansoura university (VM.R.21.215). An informed consent was received from the farm owner about the proposed treatment protocol and the farmer was given information about the potential beneficial effects of the products being used.

Study design and allocation

The study calves were randomly allocated into five equal sized groups (five animals each). The first group was supplemented daily with 10 ml of Super Immune® (Mapco Pharmaceutical industries, Egypt contains 100,000 IU vit. A, 50mg vit E and 10mg vit. C, Eucalyptus oil, Lavender oil and Peppermint oil). The second group was supplemented with 100,000 IU Vit A (Aviton, Kahira pharmaceutical & Chemical Industry Company, Egypt) and given intramuscularly. The third group was supplemented with Vit. E (Vitaselen, Adwia Pharmaceuticals Company, Egypt) at a dose rate of 6.66 IU/kg BW subcutaneously; and the fourth group was supplemented with Vit. C (Cevaryl, Memphis Co for pharmaceutical and Chemical industries, Cairo Egypt) at a dose rate of 30 mg/kg intravenously. The former formula was given orally for five consecutive days, while the later supplements were given twice with 72 hours apart. Calves of the fifth group received a sterile saline solution (0.9 % NaCl) and served as control group.

Following up protocol

The study cows were clinically monitored throughout the study period. The vital signs were routinely monitored prior to, and during, the study. The body weight for each calf was also recorded pre-treatment and weekly for 6 weeks post-treatment using a digital weighing scale.

Blood samples

Ten mL of blood were drawn from each calf via jugular venipuncture at the following time points: pre-treatment (T0), three

weeks post treatment (T3) and six weeks post treatment (T6). The collected blood was collected in plain tubes (i.e. without anticoagulants) to yield serum. Only clear non-hemolyzed sera were harvested and aliquoted for biochemical analyses of malondialdehyde (MDA), Total antioxidant capacity (TAC), glutathione reduced (GR), Glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) which were measured spectrophotometrically (Photometer 5010, Germany) following standard methods using commercially available test kits (Biodiagnostics, Cairo, Egypt).

Statistical analysis

All statistical analyses were carried out using a commercial software program (GMP SAS for windows, USA). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. Data were found normally distributed; therefore, results were presented as mean \pm SD. Then, a general linear model with repeated measure ANOVA was used to assess the effect of within group (time), between groups (treatment), and time x treatment interaction. For this purpose, Wilks' Lambda was used. Whenever Wilks' Lambda test was found significant ($p < 0.05$), one-way ANOVA with post hoc Tukey's HSD test was used at each time point to assess which group was significantly different. For all analyses, result was considered significant at $p < 0.05$.

RESULTS

All the study calves were clinically healthy and showed no detectable signs of illness. All vital signs were within the normal reference intervals and the calves remained healthy throughout the study period. No evidence of gastrointestinal abnormalities was also documented. Results of the present experiment are shown in Figures 1-4.

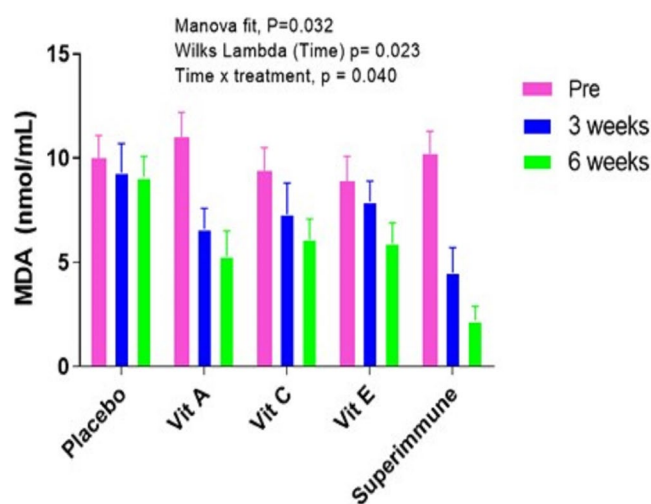


Fig. 1. Plasma MDA in calves supplemented with Super Immune® versus single use of vit. A, E and C.

Calves received a combination of vitamins and volatile oils showed a significant ($p < 0.05$) increase of the body weight compared with control group at 6 weeks post-treatment; however, no-significant variations were recorded compared with supplementation of a single nutrient. There was also a significant increase of TAC (MANOVA fit: $p < 0.05$, Wilks' Lambda, $p < 0.01$), GR (MANOVA fit: $p < 0.05$, Wilks' Lambda $p < 0.05$) and GST (MANOVA fit: $p < 0.05$, Wilks' Lambda: $p < 0.05$) in calves received the combi-

nation of vitamins and volatile oils compared with other groups. However, there was a significant decrease of MDA levels (MANOVA fit: $p < 0.01$, Wilks' Lambda $p < 0.01$). On the other hand, there was non-significant increase of CAT (MANOVA fit: $p < 0.55$, Wilks' Lambda: $p = 0.072$) and SOD (MANOVA fit: $p < 0.067$, Wilks' Lambda: $p = 0.065$) in calves received the combination of vitamins and volatile oils compared with other groups.

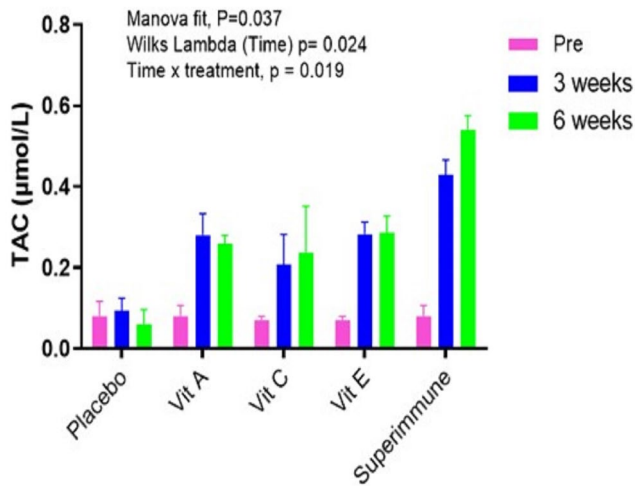


Fig. 2. Plasma total antioxidant capacity (TAC) in calves supplemented with Super Immune® versus single use of vit. A, E and C.

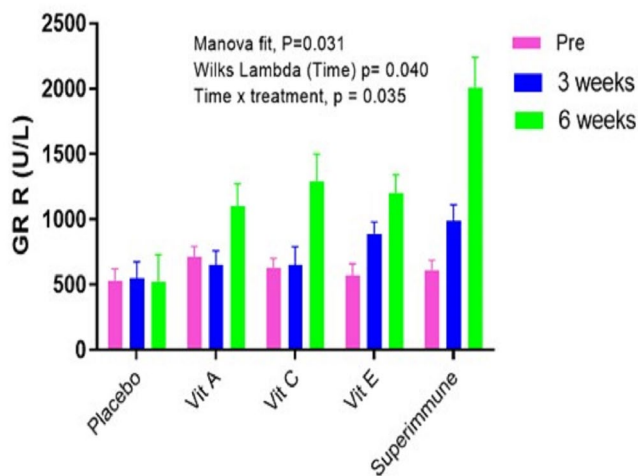


Fig. 3. Plasma GR in calves supplemented with Super Immune® versus single use of vit. A, E and C.

DISCUSSION

Up to now, there has been scarce scientific data that support the efficacy of essential oil mixtures either alone or in combination with other nutrients and the cumulative evidence is not always sufficient (Nehme *et al.*, 2021). In the present study, orally administered a combination of vitamins and volatile oils was found to be superior to that of single nutrient in promoting weight gain in calves. As shown in previous studies, the influence of volatile oils or supplementing a single nutrient on animal performance may vary. In that context, some authors have reported a positive effect of supplementing an essential oil mixture given at different doses during peri-weaning period (Soltan, 2009). In that study, it has shown that the preweaning supplementation of essential oil mixture can improve feed conversion ratio daily body gain but had no effect on body gain while post weaning supplementation can increase daily body weight, reduce feed intake, improve feed

conversion rate while the higher dose of essential oil mixture was less effective and uneconomical to be used. In another study, it has shown that administration of vitamin A (alone or in combination with iron) has improved weight gain and yielded higher amounts of RBC parameters than the control calves (Moosavian *et al.*, 2010). However, over supplementation with ascorbic acid to calves did not alter the total weight gains but, it increased the weight gain of the female calves compared with female calves of control group (Seifi *et al.*, 2010). Interestingly, in a recent study, it has been documented that Vitamin E/Se supplementation to pregnant ewes had resulted in a significant increase of the body-weight of the lambs at birth compared to lambs born to non-supplemented ewes (El-Sayed *et al.*, 2020).

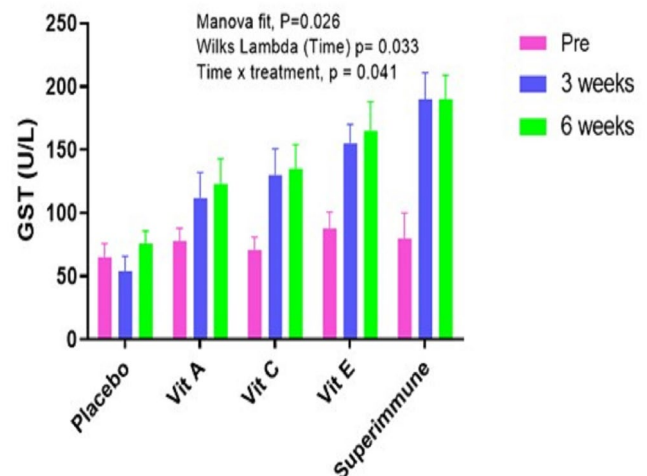


Fig. 4. Plasma GST in calves supplemented with Super Immune® versus single use of vit. A, E and C.

Actually, the antioxidative properties of EOs have been investigated mainly from human perspectives. However, the available literature is being handful and appears to be promising (Nehme *et al.*, 2021). In the present study, supplementation of a combination of vitamins and EO mixture has significantly altered the redox status as indicated by increasing antioxidant parameters (including TAC, GR, and GST) and decreasing markers of oxidative stress (i.e. MDA) compared with other groups. In one study, it has shown that injection of vitamin E could provide beneficial effects to plasma antioxidant activity and MDA values. Therefore, injection may be the best method of vitamin E administration in newborn calves for protecting them in the stressful postnatal condition (Mokhber-Dezfouli *et al.*, 2008). On the other side, it has been revealed that higher enzyme levels of the antioxidative enzymes, particularly SOD and glutathione peroxidase (GPx), were found in ageing rats dietary supplemented with thyme oil or thymol (Youdim *et al.*, 2000). However, the antioxidant activity of *O. vulgare* EO has been reported to be less effective than Vitamin C, but comparable with the Vitamin E (Kulicic *et al.*, 2004). Many studies exploring the bioactivity of EOs have mainly attributed their antioxidant capacity to terpenoids with phenolic groups such as carvacrol, methyl chavicol, thymol, and eugenol (Mimica-Dukić *et al.*, 2016). In general, it has been reported that most EOs have the virtue of being non-toxic, residue-free, and thought to be ideal growth promoters for both milk and beef production and quality (Amorati *et al.*, 2018).

CONCLUSION

The results of the present study indicated that a combination of vitamin A, E and C and volatile Eos (Super Immune®) may have a better effect on body weight gain and antioxidant status in suckling calves than supplementation with single vitamin. Use of combinations of antioxidant vitamins and volatile oils in growing newly born calves has beneficial effect on the healthy status

and oxidant/antioxidant balance.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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