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Ameliorative Effect of *Aloe vera* Supplementation in Poultry Feed

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ABSTRACT

Restricted use of chemical antioxidants and antibiotics as growth enhancers in poultry diet has led to open channels for herbs as natural feed additive in current times. Scientists try to find novel herbal feed additives which are free from toxic effects and exhibit increase in performance of poultry birds. *Aloe vera* is a well-known herb characterized by antioxidant, anti-inflammatory, immunostimulatory and growth promoting properties. The therapeutic potential of *Alo vera* is attributed to its rich phytochemistry. The current study was designed to evaluate the ameliorative potential of alcoholic extract of *Aloe vera* (family *Liliaceae*) in poultry birds**.** The birds were divided in four groups of six birds in each group. Group I: Contol (C), Group II: treatment I (T_1), Group III: treatment II (T_2) and Group IV: treatment III (T_3). *Alo vera* supplementation was given in three treatment groups at the dose of different concentrations (T1: 2gms, T2:5gms and T3: 7gms). Blood samples were collected on different time points 0, 14, 28, 42 and 56thdays and analyzed for different parameters. The altered biochemical parameters due to oxidative stress like Lipid peroxidation (LPO), super oxide dismutase (SOD), reduced glutathione (GSH), glucose -6-phosphate dehydrogenase (G6PD), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT),blood urea nitrogen (BUN) and Creatinine were significantly ameliorated to a great extent by *Aloe vera* supplementation. The alcoholic extract of *Aloe vera* showed potent anti-oxidant and hepato-protective activity in poultry birds.

Keywords: Oxidative stress, AST, ALT, KFT, *Aloe vera,* poultry, herb

Herbal feed additives are commonly defined as plants, plant extracts and plant derived pure compounds used into poultry feeds. These feed additives enhance the performance of poultry birds due to their ameliorative effects on poultry birds through strengthening their immune system against the diseases and increased productivity, performance and quality of the products obtained from these birds. Various plant derived products have been studied in terms of their ameliorative potential in poultry. Oxidative stress has deleterious effects on health. It leads to the hyper-production of reactive oxygen species (ROS) which inturn leads to multifactorial illness including weakness of immune system, decrease in performance and increased susceptibility to diseases. Natural antioxidants have gained tremendous importance in current era of world because the synthetic antioxidants possess toxic effects. Thus efforts are being made to search for natural products from plants. *Aloe vera* is one of the most popular and commonly found herb belongs to the family *Liliaceae* has been used since immemorial times as a natural medicine for various disorders (Christaki and Florou-Paneri, 2010; Ahlawat and Khatkar, 2011). Farmers in rural areas use *Aloe vera* for prevention and treatment of disease (Mawale *et al*., 2005). Feeding of different types of herbs as feed supplements has decreased oxidative stress and increase productive performance of birds (Bashir *et al.,* 2014). Cinammon and pepper improved the digestibility of various nutrients in chicken (Hernandez *et al.,* 2004). *The* rich phytochemistry of *Aloe vera* has shown a great potential in performance of poultry birds in terms of increase in body weight (Alemi *et al.,* 2012), increase in immune status by increasing by increasing humoral and

cellular immunity (Shokorena *et al.,* 2012; Besharatain *et al*., 2012). *Aloe vera* contains more than 200 compounds including oloesins, anthraqinones, acemannan, sterols, saponins, vitamins, chromone, aloe verasin and hydroxyaloin. *Aloe vera* as a natural antioxidant is more potent than the synthetic butylated hydroxyl toluene (BHT) (Yun *et al.,* 2003). *Aloe vera* has potent antioxidant properties against pro-oxidant induced membrane damage and cellular damage by reduction in levels of cytochrome p_{50} and cytochrome b_5 besides this it also has anticancer, antiulcer, antiviral and immunomodulatory properties. Due to its immense ameliorative potential, therapeutic properties and minimum toxic effects the current study was performed to evaluate its ameliorative potential as a feed supplement in poultry

MATERIALS AND METHODS

The present study involved 24 Jabalpur color birds of 32 weeks age. The experiment was approved and carried out at Nanaji Deshmukh Veterinary Science University Jabalpur, Madhya Pradesh, India. The birds of different groups were kept separately in individual cages and maintained under similar hygienic conditions. All birds were provided basal diet having 2700 ME Kcal kg^{-1} feed and 17% protein. These birds were divided in four groups of six birds each. One group was kept as control (C) and provided basal diet without any herbal supplement.The other three groups (T_1, T_2 and T_3) of birds were given *Aloe vera* supplementation at the rate of 2gm, 5gm and 7gm respectively along with basal diet. Blood samples were collected on 0, 14, 28, 42 and 56 day of experiment in clot activator vials and serum was obtained after centrifugation at 5000 RPM. Serum samples were used for biochemical assays. Lipid peroxidase was estimated according to the method of Rehman (1984). Reduced glutathione was estimated as suggested by Prins and Loos (1969). SOD activity was determined by the method of Madesh and Balasubramanian (1998). Serum G6PD was estimated by the method of Bauman *et al.* (1970).

All other parameters (BUN, Creatinine, AST, ALT and ALP) were assayed using commercially standard diagnostic kits.

Table 1: Blood LPO (µM/l), SOD (Units/g Hb), GSH (µM/l) and Serum G6PD (IU/L) in birds fed with Aloe vera at different intervals and doses

		Duration				
	Groups	**0 Day**	**14 Day**	**28 Day**	**42 Day**	**56 Day**
LPO	C	$3.09^{1}\pm0.02$	$3.10^{1}\pm0.02$	$3.09^{1}\pm0.17$	$3.08^{1}\pm0.016$	3.12 ± 0.016
	T_1	$3.18^{b;2}\pm0.02$	$3.17^{b;2}\pm0.01$	$3.16^{b;2}\pm0.016$	$3.11^{ab;1,2}\pm0.02$	$3.10^{a}\pm0.19$
	T_2	$3.16^{b;2}\pm0.09$	$3.15^{b;1}\pm0.08$	$3.13^{ab;1,2}\pm0.01$	$3.11^{ab;1,2}\pm0.01$	$3.09^{a}\pm0.08$
	T_3	$3.20^{b;2}\pm0.10$	$3.19^{b;2}\pm0.01$	$3.17^{b;2}\pm0.06$	$3.14^{ab;2}\pm0.01$	$3.11^{a}\pm0.10$
SOD	C	$297.0^{1}\pm0.966$	$296.5^{1}\pm0.764$	$296.83^{1}\pm0.94$	$297.5^{1}\pm1.05$	$297.83^{1}\pm0.87$
	T_1	$298.33^{a;1}\pm0.66$	$299.67^{a;2}\pm0.76$	$300.67^{ab;2}\pm0.80$	$301.67^{b;2}\pm0.80$	$302.83^{b;2}\pm0.87$
	T_2	$302.5^{a;2}\pm0.76$	$305.33^{b;3}\pm0.76$	$306.16^{b;3}\pm0.87$	$306.83^{b;3}\pm0.79$	$307.67^{b;3}\pm0.76$
	T_3	$303.831^{a;2}\pm0.87$	$306.831^{b;3}\pm0.30$	$309.17^{b;4}\pm0.30$	$311.17^{bc;4}\pm0.60$	$312.17^{c;4}\pm0.47$
GSH	C	10.42 ± 0.20	$10.67^{1}\pm0.33$	$10.38^{1}\pm0.26$	$11.00^{b;1}\pm0.43$	$10.75^{b;1}\pm0.17$
	T_1	$11.17^{a}\pm0.95$	$23.33^{b;2}\pm0.92$	$24.33^{b;2}\pm0.80$	$47.83^{c;2}\pm1.56$	$53.50^{d;2,3}\pm1.02$
	T_2	$11.33^{a}\pm0.84$	$31.17^{b;3}\pm2.27$	$35.13^{b;3}\pm1.70$	$52.17^{c;2,3}\pm2.08$	$59.83^{d;3}\pm1.74$
	T_3	$11.33^{a}\pm1.26$	$31.83^{b;3}\pm1.30$	$35.5^{c;3}\pm3.29$	$54.83^{d;3}\pm2.73$	$61.83^{e;3}\pm2.09$
G6PD	C	32.50 ± 0.11	$32.38^{1}\pm0.08$	$32.54^{1}\pm0.03$	$32.43^{1}\pm0.05$	$32.56^{1}\pm0.03$
	T_1	$30.40^{a}\pm0.05$	$34.59^{b;2}\pm0.15$	$35.42^{c;2}\pm0.14$	$36.55^{d;2}\pm0.14$	$38.41^{e;2}\pm0.12$
	T_2	$32.41^{a}\pm0.05$	$35.48^{b;3}\pm0.14$	$36.47^{c;3}\pm0.13$	$38.48^{d;3}\pm0.12$	$40.43^{e;3}\pm0.12$
	T_3	$32.42^{a}\pm0.01$	$36.58^{b;4}\pm0.11$	$38.51^{c;4}\pm0.15$	$40.51^{d;4}\pm0.12$	$41.40^{e;4}\pm0.13$

Mean ±SE with different alphabets and numbers in superscripts vary significantly ($P<0.05$) in a row or parameter wise in a column, respectively.

Table 2: Serum AST (mg/dl), Serum ALT (IU/L), Serum creatinine (mg/dl) and BUN (mg/dl) in birds fed with Aloe vera at different intervals and doses

		Duration				
	Groups	0 Day	14 Day	28 Day	42 Day	56 Day
AST	C	164.13 ±0.84	165.53±0.15	164.60±0.30	164.67±0.39	165.12^{1}±0.52
	T_1	165.34±0.24	165.37±0.15	164.62±0.17	164.16±0.26	164.65^{2}±0.44
	T_2	164.79^{b}±0.52	165.52^{b}±0.15	164.52^{ab}±0.42	163.61^{a}±0.32	$163.41^{a;2}$±0.65
	T_3	164.68±0.52	165.50±0.16	164.27±0.41	164.87±0.57	164.64^{2}±0.34
ALT	C	36.89±0.14	35.78^{2}±0.34	36.44^{1}±0.57	36.21±1.00	36.55^{1}±0.30
	T_1	36.50^{b}±0.17	$34.80^{a;2}$±0.55	$40.37^{b;2}$±0.51	37.73^{b}±0.91	$37.30^{b;1,2}$±0.45
	T_2	36.62^{b}±0.19	$33.56^{a;1,2}$±0.52	$39.95^{c;2}$±0.96	37.07^{bc}±0.24	$36.37^{b;1}$±0.29
	T_3	35.78^{b}±0.22	$33.03^{a;1}$±0.53	$39.33^{c;1,2}$±0.56	37.03^{bc}±0.28	$38.27^{c;1,2}$±0.59
Creatinine	C	0.81±0.04	0.84±0.01	0.855±0.08	0.84±0.011	0.84±0.014
	T_1	0.842±0.012	0.847±0.009	0.845±0.014	0.84±0.066	0.845±0.008
	T_2	0.81±0.024	0.814±0.02	0.847±0.008	0.843±0.011	0.845±0.008
	T_3	0.845±0.03	0.848±0.009	0.835±0.014	0.848±0.05	0.848±0.011
BUN	C	3.70±0.05	3.65±0.06	3.60±0.05	3.78^{2}±0.09	3.85^{2}±0.08
	T_1	3.75±0.04	3.65±0.02	3.85±0.08	3.70^{2}±0.06	3.88^{2}±0.11
	T_2	3.65^{ab}±0.11	3.75^{ab}±0.06	3.80^{b}±0.07	$3.72^{ab;2}$±0.06	$3.50^{a;1}$±0.17
	T_3	3.77^{b}±0.14	3.70^{b}±0.08	3.62^{ab}±0.12	$3.43^{a;1}$±0.07	$3.47^{ab;1}$±0.14

Mean±SE with different alphabets and numbers in superscripts vary significantly (P<0.05) in a row or parameter wise in a column respectively.

Stastical Analysis

All the data were analyzed using the CRD (completely randomized design) by one way ANOVA procedure of SPSS software. Duncan's multiple range test was used to compare the differences among treatment means.

RESULTS AND DISCUSSION

The data presented in table 1 revealed that significant changes were recorded in all treatment groups and intervals in LPO activity. Lipid peroxidation is the key indicator of oxidative stress response and its status is measured by increase in concentration of malonaldehyde (MDA). The increase in concentration of MDA is directly proportional to increase in excessive reactive oxygen species (ROS) (Sharbidre *et al*., 2011). The decrease in MDA levels was significant in treatment groups on days 56, as compared to the control. Antioxidant enzymes like SOD and GSH are combating oxidative stress by scavaging the free radicals increased by oxidative stress (Kohen and Nyska 2002). SOD activity was significantly high on all treatment groups in dose dependent manner. The highest activity was found in T_3 on day 56. Maximum increase in GSH level was observed on 56th day in T_3 group. Mean values obtained in all treatment groups were significantly high as compared to control group at all days of observations. The G6PD activity was elevated continuously in all periods of study at different doses. On day 0 the values were similar, however, the increase followed the pattern $T_3>T_2>T_1$ in all periods of investigations and maximum value was found on 56th day in all groups. *Aloe vera* enhances the body's natural defenses against oxidative stress by increasing the amount and level of activity of the body's natural antioxidant enzymes such as liver catalase, SOD and G6PD (Singh *et al*., 2000; Saada *et al*., 2003).

Aloe vera in various formulations has been shown to reduce oxidative stress and markers of oxidative stress in human clinical experiments (Bloomer *et al.,* 2010). There is an

inverse relationship between the percent thiobarbituric acid reactive substances and per cent inhibition of peroxidation (Pritam and Kale, 2007). If the per cent inhibition of peroxidation is high, antioxidant activity is also high. The extract from the aged leaves exhibited strongest radical scavenging activity **(**Amareswari *et al*., 2012) that directly inhibit the chemical reactions that produce oxidative molecules causing damage (Wu *et al.,* 2006). Previously published papers report that *Aloe vera* gel increased the GSH concentration by four times in diabetic rats thus had significant cardio-protective activity (Jain *et al*., 2010). Also, significantly increased GSH and SOD and decreased levels of lipid peroxidation (LPO) and hydroperoxides in tissues of diabetic rats had reverted back to near normal levels after treatment with *Aloe vera* gel extract (Rajasekaran *et al.,* 2005). In contrast, GSH level increased significantly with *Aloe* pulp and gel extracts in comparison to diabetic control group in Type 2 diabetes mellitus (T2DM) rats and found reduced degenerative changes in the, liver tissue, LPO with the *aloe* pulp and gel extracts in comparison to diabetic control group (Can *et al.,* 2004). Lim *et al*. (2003), showed anti-oxidative and hypocholesteremia effects of *Aloe vera* in the liver and proposed that a life-long intake of *Aloe vera* had superior anti-oxidative action against lipid peroxidation *in vivo*. This was indicated by reduced levels of hepatic phosphatidylcholine hydroperoxide and elevation of the SOD activity up to the normal level.

Liver specific enzymes were also studied to evaluate the hepatoprotective effect of *Aloe vera* supplementation in the diet of birds. AST and ALT are being considered as liver specific enzymes in liver injury as these enzymes leach out from hepatocytes in liver disorders. Highly significant variation was recorded among the time intervals in AST and ALT activity. Significantly low activity of AST and ALT was found in all periods in T_3 group. These findings are in agreement with previously reported studies Rajasekaran *et al.* (2006) and Rehman *et al.* (2011), reported that oral administration of *Aloe vera* extract, significantly decreased AST and ALT activity in the liver of diabetic rats. However, Bolkent *et al*. (2004) and Yagi *et al.* (2009), did not find any change in serum AST, ALT activities on administration of *Aloe vera* indicating sits safety to liver. Reduction in the activity of these enzymes indicates the healthy status of liver of birds.

The nitrogenous compounds urea and creatinine are eliminated from the body by the kidneys. The excretion of waste product of the body is minimized indicating maximum utilization of metabolites which promotes an increase in weight and production of more eggs. No significant variation was recorded in serum creatinine. In T_2 and T_3 group the reduction in BUN was significant on the 56^{th} day and in T_3 on the 42^{nd} day. No changes in levels of BUN and creatinine were observed by Wattanasrisin (1988). The same results were shown with fresh and preserved *aloe vera* gel supplementaion by Jirakulchaiwong *et al*. (1991). However, treatment with *Aloe vera* in streptozotocin-induced diabetic rats decreased level of urea and increased the level of serum protein as compared with diabetic group in previously reported studies (Rajasekaran *et al*., 2004). Our results can be supported by Yan *et al.* (2014) that *Aloe vera* has potent hepatoprotective potential against the chronic alcohol liver diseases by reducing MDA, increasing SOD and GSH and other antioxidant enzymes. Changkang *et al*. (2007), also showed increase in feed efficiency by inclusion of *Aloe vera* in broiler diet.

CONCLUSION

The abrogation of oxidative damage caused by reactive oxygen species (ROS) and free radicals is major area of study now a days. Synthetic antioxidants although provide protection and increased performance in poultry birds yet they are full of side effects and face resistance. *Aloe vera* supplementation in the current study has shown its protective potential by decreasing the lipid peroxidation, increasing the antioxidant status and providing protection to the vital organ like liver and kidney. *Aloe vera* can be considered as best substitute to chemical antioxidants, liver tonics and feed additives in poultry. However further studies are warranted to explore its efficacy and mechanism of action.

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