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EFFECT OF ZINC FORTIFIED EDIBLE COATED APRICOTS ON HEMATOLOGY IN RABBITS

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ABSTRACT

Worldwide, the zinc deficiency has appeared as one of the alarming issues amongst the poor nations. In current exploration, apricots were applied with edible coatings (chitosan and alginate) @ 1 and 2% along with fortification with zinc sulfate and zinc chloride @ 30 & 50 ppm concentration. The experimental diets along with a control were fed to rabbits (150 g/day/rabbit) along with normal diet in a completely randomized design for a period of 56 days. The consumption of zinc fortified chitosan and alginate coated apricots imparted substantial effect on immunity level of the body. The T-lymphocytes and hemoglobin of experimental rabbits were significantly improved by the provision of zinc fortified apricots with maximum T-lymphocytes and hemoglobin were observed in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 22.34±1.29 & 13.07±0.53 % followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 22.21±1.35 & 12.94±0.47, 22.02±1.14 & 12.78±0.45 and 21.50±1.14 & 12.61±0.51 % as comparison to G_0 by 21.22±1.13 & 12.32±0.36 %, respectively. From current findings, it can be concluded that zinc fortification through edible based coating is a pragmatic approach to triumph over hidden hunger with special reference to zinc.

Keywords: Zinc fortification, Edible coatings, Chitosan, Alginate, Apricot

INRODUCTION

Recent era has witnessed the coinage of various dietary interventions aimed at combating malnutrition with special reference to hidden hunger. The micronutrients are regulating various metabolic pathways and their deficiencies lead to physiological abnormalities that may hamper the health stratum and life quality of the individuals (Prasad, 2012). In an effort to curtail the challenges by such diet linked maladies, various strategies have been devised. Amongst, fortification has proven as a vibrant, far reaching and effective choice to overcome the consequences associated with Zn deficiency. Additionally, this rationale is flexible and socially acceptable to improve the nutrients balance by incorporating certain deficient micronutrients in food (Wang *et al.*, 2002).

Zinc deficiency in human body is linked with numerous malfunctions like diabetes mellitus, immune dysfunction and liver necrosis. Its application has proven effective to improve glycemic response in type 1 and 2 diabetes. Zinc put forth sound effect by sustaining the signal transduction of insulin and reducing the generation of cytokines, leads to β-cell death during the inflammatory process in the pancreas (Prasad, 2012). In avoidance to lower amount of zinc in the body, a mobile pool is mandatory for its distribution. Additionally, body zinc level is regulated by transporters and zinc binding proteins like metallothionein (Capdor *et al*., 2013).

MATERIALS AND METHODS

The current research work was carried out at the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF), Pakistan. In the present research, locally available apricot variety (Sufeda) was used for zinc fortification through alginate and chitosan based edible coatings. Furthermore, the storage behavior of coated apricots was assessed followed by bioefficacy trial. The materials and protocols followed are elaborated herein.

Procurement of raw materials: Selection of fresh apricots was made on the basis of uniformity in size, shape, color and absence of physical damage.

Preparation of whole fruit: The collected apricots were washed with water to loosen the dirt and grits adhered to the surface of fruit in the Canning Hall of the NIFSAT, University of Agriculture Faisalabad. Afterwards, the apricots were randomly assorted into lots and stored at 4-6 ^oC to avoid browning and undesirable biochemical changes before further treatment.

Application of edible coatings: After the development of zinc fortified edible coatings *i.e.* alginate and chitosan with their two levels 1 and 2% containing fortificants *i.e.*

$ZnSO_4$& $ZnCl_2$ @ 30 & 50 ppm of each were applied on different lots of apricots through dipping (Table 1). Later, the coated apricots were allowed to dry for 15-20 min.

Efficacy studies: To evaluate the bioavailability of zinc fortificants, a model feed trial was performed. For the purpose, 115 rabbits were procured and housed in the Animal Room of National Institute of Food Science and Technology. The rabbits were acclimatized on basal diet for the period of seven days under controlled conditions. The age of selected rabbits ranged between 11 to 12 months while the weight varied from 836 to 849g. The temperature (23±2 ºC) and relative humidity (55±5%) was maintained throughout the experiment with 12 hr light-dark period. Before the initiation of trial, baseline values for selected parameters were established. During 56 days study span, the rabbits were randomly divided into five groups, ten in each and provided with selected uncoated (control) and zinc fortified apricots (150 g/day/rabbit) along with normal diet (Table 2). For the collection of blood samples in rabbits, arterial blood sampling was done by removing the hairs from the ear and blood was taken from the marginal ear vein. The blood samples were collected from the overnight fasted rabbits at 0, 15th, 30th, 45th and 60th day of modeling. The collected blood samples were analyzed for hematological parameters with special reference to red and white blood cells indices.

Table 1: Study plan for the development of zinc fortified edible coatings.

Coating Type	Fortificant	Treatments	Coating (%)	Fortificant level (ppm)
Control	-	T_0	-	-
Alginate	**$ZnSO_4$**	T_1	1	30
		T_2	1	50
		T_3	2	30
		T_4	2	50
	$ZnCl_2$	T_5	1	30
		T_6	1	50
		T_7	2	30
		T_8	2	50
Chitosan	**$ZnSO_4$**	T_9	1	30
		T_{10}	1	50
		T_{11}	2	30
		T_{12}	2	50
	$ZnCl_2$	T_{13}	1	30
		T_{14}	1	50
		T_{15}	2	30
		T_{16}	2	50

Hematological analyses: Red blood cells indices with special reference to total red blood cells (TRBCs) and hemoglobin (hb) were estimated. Likewise, white blood cell indices including monocytes, T-lymphocytes, b-lymphocytes, eosinophils and neutrophils were measured by using Automatic Blood Analyzer (Nihon Kohden, Japan) (AlHaj *et al.*, 2011).

Table 2. Diet plan used in the *in vivo* studies.

Groups	Diet plan
G_0	Control (unfortified apricots)
G_1	Apricots coated with 2% alginate containing 50 ppm $ZnSO_4$
G_2	Apricots coated with 2% alginate containing 50 ppm $ZnCl_2$
G_3	Apricots coated with 2% chitosan containing 50 ppm $ZnSO_4$
G_4	Apricots coated with 2% chitosan containing 50 ppm $ZnCl_2$

Statistical analysis: The collected data were analyzed statistically by applying two way factorial under completely randomized design (CRD) at 5% level of significance following the guidelines of Steel *et al.* (1997).

RESULTS AND DISCUSSION

Hematological aspects: Mean squares demonstrated that T-lymphocytes, b-lymphocytes, neutrophils and hemoglobin of experimental rabbits affected significantly due to treatments and time intervals however, non-significant behavior for leukocytes, monocytes and eosinophils was observed (Table 3).

Table 3. Means squares for hematological aspects of rabbit's blood.

S.O.V	df	T-lymphocytes	Leukocytes	b-lymphocytes	Monocytes	Eosinophils	Neutrophils	Hemoglobin
Groups (G)	4	3.45379*	2849.15^{NS}	4.83116*	0.00605^{NS}	0.00291^{NS}	4.77692*	1.27513*
Duration gap (D)	4	2.52948*	5594.58^{NS}	8.53510*	0.00629^{NS}	0.00317^{NS}	3.87307*	0.23167*
D x G	16	0.19589*	113.89^{NS}	0.56605*	0.00052^{NS}	0.00018^{NS}	0.07292*	0.01774*
Error	50	0.03405	23.09	0.00383	0.00012	0.00009	0.00438	0.00055

($p<0.05$); * = Significant; ^{NS} = Non significant

The Table 4 elucidated highest value for T-lymphocytes at 60 days in group G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 23.06±1.46% followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 22.78±1.31%, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 22.41±1.12% and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 21.74±1.24%, respectively whilst, lowest in G_0 (control) 21.44±1.09%. As the time period of rabbit modeling proceed, the hematological parameters were also significantly influenced as mentioned in the respective mean tables. The groups G_1 and G_3 were noticeably affected as compared to G_2 and G_4. The reason for this significant affect was the bioavailability of the fortificant ($ZnSO_4$) in G_1 and G_3 in contrast to G_2 and G_4 having $ZnCl_2$.

The observed values for leukocytes at 60 days were 7264.00±73.79, 7261.00±75.03, 7253.00±74.73and 7261.00±75.03cu mm in G_3, G_1, G_4 and G_2 groups, respectively whereas, 7216.67±75.13cu mm in G_0 (Table 5). Contrarily, the highest value of b-lymphocytes at 60 days was noticed in G_3 trailed by G_1, G_4 and G_2 as 48.42±2.51, 48.32±3.14, 47.23±2.18 and 47.15±2.49%, respectively although, lowest in G_0 45.94±3.17% (Table 6). The treatment groups that were fed on 2% chitosan + 50 ppm $ZnSO_4$ (G_3) showed maximum improvement in hematological attributes followed by 2% alginate coating + 50 ppm $ZnSO_4$, 2% chitosan coating + 50 ppm $ZnCl_2$, 2% alginate coating + 50 ppm $ZnCl_2$ as comparison to G_0.

The results in Table 7 reflected that values for monocytes at 60 days in group G_3, G_1, G_4, G_2 and G_0 were 1.83±0.02, 1.81±0.01, 1.79±0.03, 1.77±0.04 and 1.75±0.06%, respectively. Similarly, values for eosinophils at 60 days in G_3, G_1, G_4 and G_2 groups were 1.10±0.09, 1.08±0.03, 1.07±0.10 and 1.05±0.02%, respectively whilst, 1.05±0.01% in G_0 (Table 8).

Table 4. Effect of zinc fortified apricots on T-lymphocytes of rabbits (%).

Days	Groups				
	G_0	**G_1**	**G_2**	**G_3**	**G_4**
0	21.07±1.04^{c}	21.43±1.33^{ab}	21.16±1.06^{b}	21.54±1.47^{a}	21.40±1.38^{ab}
15	21.11±1.06^{c}	21.73±1.15^{a}	21.49±1.07^{bc}	21.70±1.26^{b}	21.71±1.11^{ab}
30	21.19±1.03^{c}	22.37±1.30^{b}	21.52±1.37^{bc}	22.52±1.09^{a}	22.25±1.13^{ab}
45	21.30±1.24^{c}	22.75±1.41^{ab}	21.57±1.13^{bc}	22.88±1.08^{a}	22.34±1.06^{b}
60	21.44±1.09^{c}	22.78±1.31^{ab}	21.74±1.24^{bc}	23.06±1.46^{a}	22.41±1.12^{b}

Means sharing the same letter in a column are not significantly different. T-lymphocytes effected significantly due to treatments and time intervals.

G_0: Control (Without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 5. Effect of zinc fortified apricots on leukocytes of rabbits (cu mm).

Days	Groups				
	G_0	**G_1**	**G_2**	**G_3**	**G_4**
0	7191.33±76.81	7199.33±75.03	7205.67±76.03	7215.00±74.04	7202.00±75.69
15	7198.00±75.03	7220.00±76.66	7212.00±75.57	7223.00±77.94	7212.67±73.61
30	7205.33±76.43	7239.00±76.56	7224.67±76.03	7242.67±77.21	7231.00±74.51
45	7211.00±76.11	7255.00±75.13	7239.00±77.51	7258.33±75.29	7247.00±76.00
60	7216.67±75.13	7261.00±75.03	7249.67±77.00	7264.00±73.79	7253.00±74.73

Table 6. Effect of zinc fortified apricots on b-lymphocytes of rabbits (%)

Days	Groups				
	G_0	**G_1**	**G_2**	**G_3**	**G_4**
0	45.23±3.03^{c}	45.54±3.06^{ab}	45.48±3.06^{b}	45.76±3.54^{a}	45.28±3.12^{bc}
15	45.62±4.04^{c}	46.41±3.13^{b}	46.59±3.16^{ab}	47.02±4.05^{a}	46.33±4.03^{bc}
30	45.66±3.26^{c}	46.65±3.46^{bc}	46.79±3.29^{b}	47.38±3.28^{a}	47.11±3.41^{ab}
45	45.92±3.02^{c}	47.73±2.94^{ab}	46.87±3.13^{bc}	47.87±3.61^{a}	47.16±2.98^{b}
60	45.94±3.17^{c}	48.32±3.14^{a}	47.15±2.49^{b}	48.42±2.51^{a}	47.23±2.18^{ab}

Means sharing the same letter in a column are not significantly different. The b-lymphocytes effected significantly due to treatments and time intervals

Table 7. Effect of zinc fortified apricots on monocytes of rabbits (%).

Days	Groups				
	G_0	G_1	G_2	G_3	G_4
0	1.72±0.17	1.75±0.11	1.75±0.12	1.75±0.10	1.74±0.09
15	1.74±0.15	1.77±0.14	1.76±0.11	1.76±0.16	1.75±0.13
30	1.73±0.06	1.79±0.08	1.78±0.04	1.79±0.02	1.78±0.03
45	1.74±0.08	1.81±0.12	1.76±0.17	1.82±0.16	1.79±0.13
60	1.75±0.06	1.81±0.01	1.77±0.04	1.83±0.02	1.79±0.03

Table 8. Effect of zinc fortified apricots on eosinophils of rabbits (%).

Days	Groups				
	G_0	G_1	G_2	G_3	G_4
0	1.02±0.10	1.04±0.09	1.04±0.02	1.04±0.01	1.03±0.06
15	1.04±0.01	1.06±0.03	1.05±0.04	1.08±0.08	1.05±0.06
30	1.03±0.03	1.05±0.04	1.03±0.01	1.05±0.09	1.04±0.02
45	1.04±0.08	1.07±0.07	1.06±0.04	1.09±0.02	1.06±0.05
60	1.05±0.01	1.08±0.03	1.05±0.02	1.10±0.09	1.07±0.10

Likewise, at 60 days the neutrophils count was 39.70±1.05, 39.08±2.20, 38.99±2.04 and 38.73±3.06% in G_3, G_1, G_4 and G_2, accordingly whereas, 37.77±1.10% in G_0 (Table 9). Similarly, the maximum value of hemoglobin was noticed at 60 days in G_3 group (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 13.34±0.77g/dL followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 13.17±0.72g/dL, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 12.89±0.64g/dL and G_2 (apricot containing 2% alginate coating with 50ppm $ZnCl_2$) 12.67±0.86g/dL, respectively whilst, minimum in G_0 (control) 12.45±0.69g/dL (Table 10).

Table 9. Effect of zinc fortified apricots on neutrophils of rabbits (%)

Days	Groups				
	G_0	G_1	G_2	G_3	G_4
0	$36.66±2.02^{c}$	$37.78±2.15^{a}$	$37.54±2.34^{b}$	$37.68±2.67^{ab}$	$37.53±2.58^{b}$
15	$36.69±2.76^{c}$	$37.81±2.16^{a}$	$37.62±2.84^{bc}$	$37.79±2.17^{ab}$	$37.64±1.82^{b}$
30	$36.83±2.24^{c}$	$38.08±2.19^{ab}$	$38.01±2.41^{b}$	$38.46±2.91^{a}$	$38.08±2.48^{ab}$
45	$36.87±1.37^{c}$	$38.67±1.29^{ab}$	$38.28±1.20^{bc}$	$38.83±1.46^{a}$	$38.47±1.73^{b}$
60	$37.77±1.10^{d}$	39.08±2.20	$38.73±3.06^{c}$	$39.70±1.05^{a}$	$38.99±2.04^{bc}$

Means sharing the same letter in a column are not significantly different. Neutrophils effected significantly due to treatments and time intervals.

Table 10. Effect of zinc fortified apricots on hemoglobin of rabbits (g/dL).

Days	Groups				
	G_0	G_1	G_2	G_3	G_4
0	$12.24±0.32^{c}$	$12.73±0.53^{ab}$	$12.55±0.44^{bc}$	$12.81±0.33^{a}$	$12.68±0.34^{b}$
15	$12.26±0.49^{c}$	$12.83±0.64^{ab}$	$12.57±0.72^{bc}$	$12.90±0.41^{a}$	$12.71±0.44^{b}$
30	$12.31±0.38^{c}$	$12.91±0.31^{ab}$	$12.61±0.23^{bc}$	$13.01±0.19^{a}$	$12.77±0.74^{b}$
45	$12.35±0.26^{c}$	$13.07±0.16^{ab}$	$12.64±0.64^{bc}$	$13.26±0.71^{a}$	$12.83±0.28^{b}$
60	$12.45±0.69^{c}$	$13.17±0.72^{ab}$	$12.67±0.86^{bc}$	$13.34±0.77^{a}$	$12.89±0.64^{b}$

Means sharing the same letter in a column are not significantly different. Hemoglobin effected significantly due to treatments and time intervals.

The instant results are in agreement with the findings of Archetti *et al*. (2008), who worked on the hematological aspects of rabbits and recorded that the values for T-lymphocytes, leukocytes, b-lymphocytes, monocytes, eosinophils, neutrophils and hemoglobin were in the range of 20-79%, 2.6-12.7%, 20-79%, 0.5-28%, 0.0-0.5% and 10-66% and 6.7-12.7g/dL, respectively. The results are also supported with the work of Ewuola *et al.* (2012), who documented that rabbit's blood has 60.6% of T-lymphocytes, 2.4% monocytes, 2.2% eosinophils, 34.8% neutrophils and 6.32mmol/L hemoglobin.

Previously, various bioefficacy studies have proven that hypozincemia increases the incidence of bacterial attack due to over-expression of NF-*k*B and targeted genes like IL-1β, ICAM-1 and TNF-α in Zn deficit mice. It has been noticed that Zn deficiency increases the inflammatory response resulting in reduced activity of vital organs including lung and liver. During a bioevaluation trial, Zn supplementation was found effective to mediate innate immunity by down-regulating NF-*k*B & TNF-α signaling pathway. It has also been observed that Zn provision reversed the dysregulation of immune expression thus reduces the rate of morbidity in mice (Bao *et al.,* 2010). In a biological study, comparison was made between healthy and common variable immunodeficient (CVID) patients to assess their serum Zn concentration. The lower zinc concentration was noticed in immunodeficient patients as compared to control group. It was further highlighted that lower zinc status worsens the disease condition thereby triggering the inflammatory response. Subsequently, Zn deficiency induces apoptosis in β cells and impairs antibodies & lymphocytes action in the human body (Santos-Valente *et al.,* 2012).

Similarly, zinc in the blood of experimental rabbits was measured as 11.2, 1.2 & 0.17 mg/L for serum, red blood cells and whole blood (Jenner *et al.,* 2007; Rashtchizadeh *et al.,* 2008). The anti-atherogenic effect of Zn supplementation was assessed on New Zealand white rabbits that were divided in to three groups on the basis of diet *i.e.* control, high cholesterol diet (HCD) and Zn+HCD. The data showed reduction by 20.9, 56.9, 18.89 and 2.2% in total cholesterol, triglyceride, LDL and WBC of Zn treated group, respectively. One of the probable reasons for zinc has an immune boosting effect is that; Zinc synthesizes carbonic anhydrase in the body that releases defensive bicarbonate ions, protecting the gastrointestinal system (Haase *et al.,* 2008; Faa *et al.,* 2008). Likewise, it is involved in the formation of alcohol dehydrogenase that metabolizes alcohol and down regulates ethanol-induced apoptosis within the liver cells by repressing Fas/Fas ligand pathway. Zn deficiency deactivates the T lymphocytes thus reduces the phagocytic action of macrophages, weakening the immunity. It is also involved in the formation of superoxide dismutase and catalase in liver that plays disease modulatory role (Faa *et al.,* 2008). Moreover, zinc being the cofactor of superoxide dismutase (SOD) suppresses the mishaps during pregnancy. Therefore, maternal Zn level is one of the basic requirements for the normal functioning of conceptus and trophoblast (Nriagu, 2007; Mistry & Williams, 2011).

The inadequacy of Zn results in impaired functions of NK (natural killer) cells, T & B cells, neutrophils and macrophages (phagocytosis). This situation induces lymphopenia (less lymphocyte production) hence attacked by chronic pathogens, compromising immunity (Scrimgeour and Lukaski, 2008; Blewett and Carla, 2012).

Earlier, it has been studied that Zn deficiency impairs the innate immune response by over-expressing various pro-inflammatory cytokines such as IL-1β, ICAM-1 and TNF-α thus increases injury at tissue and organ levels (Bao *et al*., 2010). The positive response of Zn has been assessed on lymphocytes against antigen attack. The intensity of immune senescence is related with Zn supplementation, enhancing the defense response of elderly people. It has been documented that zinc helps in the formation & maturation of T-cells subsequently synthesizes IgA & IgG. Similarly, Zn supplementation was also found effective in restoring the function of T helper cells, if treated three weeks before immunization whilst, side effects were detected if consumed after vaccination (Shankar and Ananda, 1998; Hasse *et al.,* 2008).

Later, an increasing trend in RBC and platelets by 33.88 and 11.4% was observed in Zn+HCD treated groups, correspondingly (Ren *et al.,* 2006). The data showed an increasing trend in hemoglobin by 25.5% in Zn+HCD treated groups. The current findings revealed the uplift in hemoglobin was 7.2% in group G_3 that were fed on apricots containing 2% chitosan coating having 50 ppm $ZnSO_4$. Basically, the incline in hemoglobin level is dependent on the concentration of zinc salts (alongside their type & bioavailability) and other diet constituents. Earlier, Evans and Halliwell (2001) recorded lower concentration of Zn in serum, erythrocytes and glutathione in zinc deprived rats. The instant results are in agreement with the work of Ozkan *et al.* (2012), found hemoglobin in the range of 147-208g/L in male and 108-175g/L in female rabbits. Similarly, Süvegová *et al.* (2004) noticed that hemoglobin of experimental rabbits was varied from 7.90 to 14.10g/dL.

One of the scientists groups, El Hendy *et al.* (2001) recorded the effects of different zinc levels *i.e.* (38mg/kg diet, control) and the concentration that developed hypozincemia (19mg/kg diet, 1/2 of control & 3.8mg/kg diet, 1/10 of control) in male and female rats. The hematological parameters were significantly affected by zinc insufficiency included hemoglobin, packed cell volume and total erythrocyte count. Similarly, serum concentrations of globulin, glucose, total protein and high density lipoprotein decreased in a dose-dependent manner. Whilst, the total leukocytes count, serum albumin, cholesterol, triglycerides, total lipids and low density lipoprotein were increased. Their results delineated that zinc deficiency has depressing effect on body growth, organ weights and hematological traits.

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