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NUTRITIVE EFFECT OF CABBAGE (*Brassica oleracea*) ON GROWTH, OBESITY, LIPIDAEMIA AND HAEMATOLOGY IN BROILER AND PULLET CHICKENS

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ABSTRACT

Efficacy of cabbage (*Brassica oleracea*) for ameliorating the adverse metabolic syndrome side-effects of genetically improved growth rate in chickens was examined. Sixty-four (64) day-old birds (32 Marshall Broilers and 32 Harco Black Pullets) were randomly assigned to groups of eight genotype-matched birds in a 2-factor layout within a Completely Randomized Design (CRD). Within genotype, each group was randomly assigned one of four diets (basal/control diet containing 0% cabbage, and, basal diet supplemented with 3%, 6% or 12% cabbage) for 4 weeks. All birds were fed an un-supplemented Broiler finisher diet from week 4 - 8. Bodyweight and Body Mass Index (BMI) were determined weekly. Blood Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cells (WBC), Red Blood Cells (RBC), neutrophils, lymphocytes, monocytes, basophils and eosinophils), Total serum Cholesterol (TC), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were determined at age 4 weeks. Irrespective of diet or age, Broilers exhibited significantly greater ($p<0.05$) body weight, adiposity, and lipidaemia than Pullets, and no interactions between cabbage and genotype were observed for the same traits. Dietary cabbage at 3%, irrespective of genotype significantly ($p<0.05$) improved body weight beyond week 5 while no significant effect was observed on body fatness as measured by BMI. Cabbage supplementation suppressed broiler eosinophil levels, indicating effects on mediators of innate immune surveillance, but did not influence any other blood haematological parameter, though Broilers exhibited higher ($p<0.05$) total WBC count and proportion (%) of WBC represented by neutrophils, basophils and eosinophils. In Contrast, Pullets exhibited higher RBC, PCV, and Hb, and lymphocyte and monocyte differential counts. Dietary Cabbage had a nutrigenetic effect on cholesterol: Reduced TC and LDL in Broilers in contrast to its effect in increasing the same lipids in Pullets. Cabbage however lowered HDL Cholesterol ($p<0.05$) in both genotypes, though the threshold of effect was higher (12%) in Pullets (3%). Thus Cabbage is effective in lowering heart disease risk through lowering of lipidaemia in Broilers, and improves bodyweight at market age (7-8 weeks) at 3% dietary supplementation level.

KEYWORDS: Cabbage, Broiler, Pullet, Nutritive, Metabolic syndrome.

INTRODUCTION

The incidence of heart disease which is measurable by arrhythmia is as high as 27% (Olkowski, 2007) in Broiler chickens, and morbid complication including sudden death syndrome (SDS) occurs at a frequency of 0.5 and 5% (Saki and Hemati-Matin, 2011), and contributes to economic losses sustained by the farmer during production .

Systematic genetic improvement in growth and productivity of modern commercial chickens (Fairfull *et al*., 1998) has unwittingly produced undesirable side effects which include a high frequency of leg problems and symptoms of metabolic syndrome, including obesity, dyslipidaemia (De Almeida *et al*., 2006) and insulin insufficiency, all of which compromise health and welfare of farmed birds, and may indeed compromise the health of humans when fatty cholesterol-rich meat from such birds is consumed, and may in addition damage public opinion of the poultry industry and demand for poultry products.

Breeding strategies including crossing and selection may be applied to uncouple desirable productivity and efficiency from the aforementioned side effects but such strategies require time and significant financial investment. As a stopgap, chemical genetics approaches which harness the modulatory effects of natural and/or synthetic chemical elements and compounds to ameliorate the metabolic syndrome symptoms are desirable. Where such modulators are effective and economical, they promise increases in health and welfare of farmed birds and may produce economic gains through higher yield and/or improved quality of meat and/or improved economics of production (improved feed efficiency).

Synthetic products such as aspirin, ibrupofen, chloroquine, paracetamol, may be effective (Tauseef *et*

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al., 2008), but consumer preference trends now point to an increasing demand for products that are raised without synthetic drugs and/or additives. The continuous use of synthetic feed supplements stimulates growth in chickens, but the residue is often difficult to decompose and may cause health problems to the human consumers (Stoker, 2007). Apart from consumer concern about the safety of consuming meat from chickens raised on supplementation of synthetic materials and products, there are also welfare issues as it concerns the birds. At certain levels, synthetic products and drugs are known to be toxic and detrimental to health. Production related health problems and welfare issues pertaining to these synthetic products may include gastro-intestinal bleeding and gut leakage in high-dose aspirin, ibuprofen and acetamorphen administration (Balog and Hester, 1991; Cyrus *et al.,* 2002; Tauseef *et al*., 2008; Al-Obaidi and Al-Shadeedi, 2010).

Natural products which are good candidates for ameliorating the metabolic disease side effects of fast growth rate in chickens would possess one or a combination of anti-obesity, anti-inflammatory, antioxidant and cholesterol lowering properties. In the ideal scenario, such products would mediate the desired outcome without adversely affecting live weight of birds at market age.

An increasing amount of evidence shows that the consumption of fruits and vegetables is, in general, beneficial to health due to the protection provided by the antioxidant compounds contained in them (Kahkonen *et al*., 1999). In fact, the presence of phytochemicals, in addition to vitamins and provitamins, has been considered of great nutritional interest in the prevention of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, ischemic and cardiovascular diseases and also in the aging process, in which oxidants or free radicals are involved (Chu *et al*., 2002; Pulido *et al*., 2000; Behl and Moosmann, 2002). Natural anti-oxidants have been shown to offer a vast array of health effects including lowering the level of blood cholesterol which is correlated with body cholesterol (Nuhulhuda *et al*., 2012) and there is now an increased focus on defining natural products that are capable of mediating the desired effect.

One such product is cabbage (*Brassica oleracea*), which contains several phytochemical compounds which act as antioxidants, stimulate detoxification enzymes, stimulate the immune system, positively affect the expression of hormones and act as antibacterial and antiviral agent. The effect of Cabbage is mediated by compounds which include isothiocyanates such as 1-isothiocyanate-(4R)-(methylsulfinyl)butane also known as sulforaphane (Guine *et al.*, 2007) which reduce inflammation, oxidative stress (Leja, *et al.*, 2010) and cholesterolemia, but have not as yet been examined in chickens. Sulforaphane, is produced when the enzyme myrosinase acts on glucopharanin in cruciferous vegetables (Guerrero-Beltrán *et al*., 2012). Studies have shown that Sulphoraphane mediates its ctyoprotective effect at least in part through its indirect antioxidant function, attributed to its ability to induce a cascade of antioxidant factors through the master transcriptional factor Nrf2, and its effects on Heme oxygenase-1, NAD(P)H: quinone oxidoreductase, glutathione-S-transferase, gamma-glutamyl cysteine ligase, and glutathione reductase among others. Cabbage also contains indole-3-carbionol, a benzopyrrole which is a breakdown product of the isothiocyanate glucobrassicin (Guerrero-Beltrán *et al*., 2012).Brassica species are reported to possess cancer preventive properties (Beecher, 1994) that have been attributed to the glucosinolates and their derived (isothiocyanate) products (Stoewsand., 1995). Flavonoids and other phenolics also contribute to this capacity (Guine *et al*., 2007). The presence of sulforaphane in cabbage confers it with anti-inflammatory properties.

In order for the phytochemical content of foods such as cabbage to be leveraged as nutriceutical agents for controlling the metabolic disease side effects of fast growth rate in birds, they must be thoroughly examined and their capacity to modulate the desired effects must be adequately verified. Because not all genotypes respond equally to the same environmental condition, of which food is a component (nutritional environment), the effect of genotype on the response to any nutriceutical agents must be considered in Nutrigenetics studies.

The current study was conducted to determine the effect of dietary cabbage (*Brassica oleracea*) on growth performance and metabolic disease side effects of fast growth rate in chickens and to determine whether the effect of cabbage is subject to genotype (G). Effect on body weight and linear growth of Broilers and Pullet chicks from hatch up to 8 weeks age. Also, Effects on inflammation as measured by haematological parameters and effects on cholesterolaemia as measured by Total Cholesterol, High Density Lipoprotein (HDL) cholesterol and low Density Lipoprotein (LDL) cholesterol in blood at four (4) weeks of age were also measured in the same birds.

MATERIALS AND METHODS

The research experiment was conducted in accordance with institutional (University of Ilorin) guidelines on humane care and use of animals in research and experimentation.

Experimental site

The research experiment was carried out at the Animal Pavilion of the Department of Animal Production, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State Nigeria (located within latitudes 7^0 45' and $9^0$30'N and longitudes $4^0$30'and 6^0 25'E).

Experimental Design:

The experiment was conducted as a Completely Randomized Design in which the effects of two factors individually and in combination were examined through a 2 x 4 (genotype classes x dietary supplemental Cabbage levels) factorial structure.

Experimental birds and housing

A total of 64 birds (32 Marshall Broiler chicks and 32 Harco Black Pullet chicks) were used for the experiment. The latter were sourced from Zartech

Hatchery, Ibadan; while the former were sourced from Zarm Farms Ilemona.

Day-old birds were housed in a ventilated poultry house in a cage, segmented into eight (8) compartments. They were raised in the cage throughout the eight week duration of the experiment.

Experimental materials

Fresh cabbage which was used as the experimental treatment was sourced from a popular market (Ipata market) in Ilorin, Kwara State, Nigeria. The cabbage was sliced then air dried for 3 days (in September 2012) after which it was ground to powder by use of a household blender.

Experimental management practice

All experimental birds were subjected to the same management practice and treatment throughout the experiment, with the exception of the feed, which varied in the level of cabbage between grouped of birds assigned to different dietary cabbage supplementation diets. All birds were exposed to continuous lighting (24 hours) throughout the course of the experiment.

Experimental feeding, treatment and design

At the onset of the experiment, day-old birds were randomly assigned into a total of eight (8) groups of genotype-matched birds such that there were four groups of Marshall and four groups of Harco chicks. The four groups within each genotype were randomly allocated to four (4) graded levels of dietary Cabbage supplement (0%, 3%, 6% and 12%) in the Broiler starter diet (23% Crude Protein, 3100 kcal). All birds were given feed and water *ad libitum* throughout the experiment. At age 4 weeks, all birds were switched to a broiler finisher diet containing no supplemental cabbage.

Data collection

Body Weight (BW): Body weight in grams (g) was taken on a weekly basis by the use of a sensitive scale.

Body Mass index (BMI): The body mass index was derived by dividing Bodyweight expressed in gram units by the square of body length expressed in cm units, and expressed in Kg/m^2 units. The body mass index is a reliable non-invasive measure of adiposity (fatness).

Haematology: The collected blood samples were subjected to the laboratory analysis of packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC) and White Blood Cell (WBC). PCV and Hb were determined using the capillary haematocrit, cyanomethamoglobin methods respectively while the haemocytometer method was used to determine both RBC and WBC counts. Differential white blood cell count analysis indicating the neutrophil, lymphocyte, monophil, basophil and eosinophil components of the WBC was also carried out.

Serum chemistry: Total cholesterol (TC), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) cholesterol levels in serum of fasted mice at age 4 weeks were determined.

Statistical analysis

The data were analysed as appropriate for 2 x 4 factorial design implemented within a completely randomized design. Mean and standard error values for each examined trait in each treatment group were determined by use of Microsoft Excel 2007, and all data were further subjected to analysis by use of the General linear model (GLM) procedure of SPSS Version 17 (IBM SPSS). Effects of Genotype, Diet, and their Interaction GxE_D were examined. The following model was specified:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk}$

Where Yijk = Rate of the dependent trait

μ= overall mean.

α_i = effect of the i^{th} genotype.

β_j = effect of j^{th} diet.

$\gamma_{ij} = \alpha\beta_{ij}$ =effect of the interaction between the i^{th} genotype and the j^{th} diet.

e_{ijk}= residual effect.

For each Factor (Genotype and Diet) in which more than two levels existed (Diet in the current study), significantly different means ($p<0.05$) were separated by use of the Duncan's Multiple Range procedure option in SPSS 16 (SPSS IBM).

RESULTS

Bodyweight

Diet (graded levels of Cabbage factor effect on Bodyweight: During weeks 1-4 in which cabbage was administered at graded levels, and in week 5 (1 week after discontinuation of the dietary cabbage supplementation), no significant ($p<0.05$) diet effect on body weight was observed (Table 1). At week 6 - 8, body weight of animals fed 3% cabbage was significantly ($p<0.05$) higher than observed for the Control (0% Cabbage) diet group, while the bodyweight of birds fed 6% and 12% cabbage fell between those of birds fed 0% and 3% cabbage (Table 1).

Genotype x Diet (graded levels of Cabbage) factor interaction effect on Bodyweight: Genotype did not significantly ($p<0.05$) interact with dietary cabbage levels to determine body weight at any stage over the 8-week experiment (Table 1).

Bodyweight effect of Graded levels of Cabbage within genotype (breed): At hatch, there was no significant ($p>0.05$) difference in body weight of treatment groups within each genotype of the chicks (Table 2). During weeks 1-4 in which cabbage was administered at graded levels, and in week 5 (1 week after discontinuation of the dietary cabbage supplementation), no significant ($p<0.05$) diet effect on body weight was observed in broilers (Table 2). At week 6 - 8, body weight of broilers fed 3% cabbage was significantly ($p<0.05$) higher than observed for the Control (0% Cabbage) diet group (3% Cabbage> 0% Cabbage, 6% Cabbage, and 12% Cabbage intermediate between groups at week 6; 3% Cabbage, 12% Cabbage > 0% Cabbage, 6% Cabbage at weeks 7 and 8) as shown in Table 2. In the pullet genotype, dietary cabbage had no significant effect on body weight at any of the levels

examined over the 8 week experiment period (Table 2), though there was a trend of increasing bodyweight with increasing dietary cabbage levels at 8 weeks age.

Body Mass Index

Diet (graded levels of Cabbage factor effect on Body Mass Index: During weeks 1-4 in which cabbage was administered at graded levels, and in week 5-7 in which dietary cabbage administration was discontinued, no significant ($p<0.05$) diet effect on Body Mass Index was observed (Table 1).

Genotype x Diet (graded levels of Cabbage) factor interaction effect on Body Mass Index: Genotype did not significantly ($p<0.05$) interact with dietary cabbage levels to determine Body Mass Index at any stage between weeks 1 and 7 of the experiment (Table 1).

Body Mass Index effect of Graded levels of Cabbage within genotype (breed): In week 2, was a significant ($p<0.05$) difference in Body Mass Index of Marshall birds 6%, 12% > 0% Cabbage, and 3% Cabbage intermediate between groups) as shown in Table 4. The difference between groups disappeared between weeks 3 and 6, and reappeared in week 7 (3%, 12%, 6% > 0% Cabbage). In the Pullet genotype, diet did not significantly determine BMI at any stage during weeks 1 – 7 of the experiment (Table 4).

TABLE 1: EFFECTS OF GENOTYPE (MARSHALL, HARCO), DIETARY CABBAGE LEVEL (0, 3, 6 AND 12 %) FROM 0-4 WEEKS AGE, AND THEIR INTERACTION, ON BODY WEIGHT.

	Breed irrespective of Diet		**Diet Irrespective of Breed**				
Trait	**Marshall ALL**	**Harco ALL**	**0% ALL**	**3% ALL**	**6% ALL**	**12% ALL**	**Breed x Diet**
Body weight week 0 (g)	59.47 ± 0.87 (32)*	41.56 ± 0.74 (32)	51.19 ± 2.73 (16)	51.69 ± 2.54 (16)	48.88 ± 2.49 (16)	50.31 ± 2.51 (16)	NS
Body weight week 1 (g)	146.45 ± 3.91 (31)*	71.91 ± 1.17 (32)	109.88 ± 10.7 (16)	108.44 ± 10.23 (16)	108.13 ± 11.05 (16)	107.87 ± 10.03 (15)	NS
Body weight week 2 (g)	342.77 ± 11.3 (31)*	134.55 ± 2.74 (31)	234.38 ± 29.79 (16)	233.88 ± 28.92 (16)	246.93 ± 30.3 (15)	240.07 ± 30.04 (15)	NS
Body weight week 3 (g)	628.74 ± 18.73 (31)*	215.81 ± 5.56 (31)	423.5 ± 59.66 (16)	400.25 ± 52.1 (16)	436.8 ± 59.4 (15)	429.93 ± 59.34 (15)	NS
Body weight week 4 (g)	967.42 ± 26.87 (31)*	299.23 ± 6.6 (30)	630.75 ± 95.11 (16)	663.6 ± 94.44 (15)	640.93 ± 88.5 (15)	620.47 ± 92.9 (15)	NS
Body weight week 5 (g)	1490.32 ± 30.62 (31)*	409 ± 9.18 (30)	928.44 ± 140.57 (16)	1007 ± 162.66 (15)	960 ± 138.39 (15)	940.67 ± 144.69 (15)	NS
Body weight week 6 (g)	1866.13 ± 42.59 (31)*	489.67 ± 10.11 (30)	1130.63 ± 176.11 (16)^{a}	1279.67 ± 207.33 (15)^{b}	1183.67 ± 176.18 (15)^{ab}	1166.67 ± 188.82 (15)^{ab}	NS
Body weight week 7 (g)	2235.48 ± 49.96 (31)*	571.17 ± 11.23 (30)	1343.44 ± 208.7 (16)^{a}	1519 ± 249.75 (15)^{b}	1399 ± 212.26 (15)^{ab}	1411.33 ± 232.98 (15)^{ab}	NS
Body weight week 8 (g)	2321.94 ± 47.06 (31)*	635 ± 10.97 (30)	1406.56 ± 212.16 (16)^{a}	1589 ± 249.78 (15)^{b}	1481.33 ± 215.12 (15)^{ab}	1498 ± 235.71 (15)^{ab}	NS

*group mean, standard error and count are presented as; means ± sem (n)\
a, b,c,d means with different superscripts within row are significantly different.

TABLE 2: BODY WEIGHT OF MARSHALL AND HARCO CHICKENS FED GRADED LEVELS OF CABBAGE (*Brassica oleracea*) FROM HATCH TO 4 WEEKS AGE.

	Marshall				**Harco**			
Trait	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**
Body weight week 0 (g)	61.25 ± 1.58 (8)[b]	60.75 ± 1.69 (8)[b]	56.75 ± 2.07 (8)[b]	59.13 ± 1.39 (8)[b]	41.13 ± 0.79 (8)[a]	42.63 ± 1.19 (8)[a]	41 ± 2.14 (8)[a]	41.5 ± 1.68 (8)[a]
Body weight week 1 (g)	148 ± 8.66 (8)[b]	145.63 ± 7.1 (8)[b]	145.75 ± 10.26 (8)[b]	146.43 ± 5.52 (7)[b]	71.75 ± 0.73 (8)[a]	71.25 ± 1.78 (8)[a]	70.5 ± 3.67 (8)[a]	74.13 ± 2.47 (8)[a]
Body weight week 2 (g)	334.13 ± 30.87 (8)[b]	339.88 ±18.37 (8)[b]	341.5 ± 25.83 (8)[b]	357.43 ± 12.8 (7)[b]	134.63 ± 2.73 (8)[a]	127.88 ± 6.08 (8)[a]	138.86 ± 7.09 (7)[a]	137.38 ± 5.74 (8)[a]
Body weight week 3 (g)	632.63 ± 52.15 (8)[b]	595.5 ± 21.21 (8)[b]	627.13 ± 45.37 (8)[b]	664.14 ± 19.27 (7)[b]	214.38 ± 6.26 (8)[a]	205 ± 17.05 (8)[a]	219.29 ± 10.52 (7)[a]	225 ± 8.4 (8)[a]
Body weight week 4 (g)	969.5 ± 76.92 (8)[b]	983.13 ± 44.13 (8)[b]	931.88 ± 58.1 (8)[b]	987.71 ± 25.36 (7)[b]	292 ± 7.89 (8)[a]	298.43 ± 18.07 (7)[a]	308.43 ± 10.38 (7)[a]	299.13 ± 16.44 (8)[a]
Body weight week 5 (g)	1450 ± 81.83 (8)[b]	1568.75 ± 47.19 (8)[b]	1431.25 ± 60.46 (8)[b]	1514.29 ± 41.85 (7)[b]	406.88 ± 16.47 (8)[a]	365 ± 22.78 (7)[a]	421.43 ± 15.61 (7)[a]	438.75 ± 8.85 (8)[a]
Body weight week 6 (g)	1781.25 ± 107.3 (8)[b]	1993.75 ± 67.77 (8)[c]	1781.25 ± 82.34 (8)[b]	1914.29 ± 58.47 (7)[bc]	480 ± 21.38 (8)[a]	463.57 ± 26.13 (7)[a]	500.71 ± 19.98 (7)[a]	512.5 ± 11.46 (8)[a]
Body weight week 7 (g)	2131.25 ± 93.51 (8)[b]	2375 ± 95.43 (8)[c]	2112.5 ± 112.9 (8)[b]	2335.71 ± 64.29 (7)[c]	555.63 ± 24.52 (8)[a]	540.71 ± 25.76 (7)[a]	583.57 ± 22.33 (7)[a]	602.5 ± 13.23 (8)[a]
Body weight week 8 (g)	2212.5 ± 83.32 (8)[b]	2448.75 ± 85.37 (8)[c]	2205 ± 113.34 (8)[b]	2435.71 ± 53.13 (7)[c]	600.63 ± 19.56 (8)[a]	606.43 ± 22.96 (7)[a]	654.29 ± 22.24 (7)[a]	677.5 ± 12.1 (8)[a]

*group mean, standard error and count are presented as; means ± sem (n)

a, b,c,d means with different superscripts within row are significantly different.

TABLE 3: EFFECTS OF GENOTYPE (MARSHALL, HARCO) , DIETARY CABBAGE LEVEL (0,3,6 AND 12%) FROM 0-4 WEEKS AGE, AND THEIR INTERACTION ON BODY MASS INDEX (BMI).

		Breed irrespective of Diet		**Diet Irrespective of Breed**				
Sn.	**Trait**	**Marshall ALL**	**Harco ALL**	**0% ALL**	**3% ALL**	**6% ALL**	**12% ALL**	**Breed x Diet**
1	BMI WK1	0.44 ± 0.01 (31)*	0.28 ± 0 (32)	0.37 ± 0.02 (16)[a]	0.36 ± 0.02 (16)[a]	0.35 ± 0.02 (16)[a]	0.35 ± 0.02 (15)[a]	NS
2	BMI WK2	0.75 ± 0.02 (31)*	0.41 ± 0.01 (31)	0.55 ± 0.04 (16)[a]	0.57 ± 0.05 (16)[a]	0.62 ± 0.05 (15)[a]	0.6 ± 0.05 (15)[a]	NS
3	BMI WK3	0.92 ± 0.02 (31)*	0.48 ± 0.01 (31)	0.71 ± 0.06 (16)[a]	0.67 ± 0.06 (16)[a]	0.71 ± 0.07 (15)[a]	0.71 ± 0.06 (15)[a]	NS
4	BMI WK4	1.11 ± 0.03 (31)*	0.53 ± 0.01 (30)	0.79 ± 0.08 (16)[a]	0.82 ± 0.08 (15)[a]	0.86 ± 0.09 (15)[a]	0.82 ± 0.09 (15)[a]	NS
5	BMI WK5	1.37 ± 0.02 (31)*	0.57 ± 0.01 (30)	0.96 ± 0.1 (16)[a]	0.99 ± 0.12 (15)[a]	1.01 ± 0.11 (15)[a]	0.94 ± 0.1 (15)[a]	NS
6	BMI WK6	1.48 ± 0.06 (31)*	0.63 ± 0.01 (30)	1.04 ± 0.14 (16)[a]	1.12 ± 0.12 (15)[a]	1.08 ± 0.12 (15)[a]	1.02 ± 0.11 (15)[a]	NS
7	BMI WK7	1.45 ± 0.04 (31)*	0.7 ± 0.01 (30)	0.98 ± 0.08 (16)[a]	1.16 ± 0.11 (15)[a]	1.1 ± 0.11 (15)[a]	1.1 ± 0.11 (15)[a]	NS

TABLE 4: BODY MASS INDEX OF MARSHALL AND HARCO CHICKENS FED GRADED LEVELS OF CABBAGE (BRASSICA OLERACEA) FROM HATCH TO 4 WEEKS AGE.

		Marshall				Harco			
Sn.	**Trait**	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**
1	BMI WK1	0.44 ± 0.02 (8)^{b}	0.44 ± 0.02 (8)^{b}	0.44 ± 0.02 (8)^{b}	0.43 ± 0.02 (7)^{b}	0.29 ± 0.01 (8)a	0.28 ± 0.01 (8)^{a}	0.26 ± 0.01 (8)^{a}	0.28 ± 0.01 (8)^{a}
2	BMI WK2	0.7 ± 0.03 (8)^{b}	0.73 ± 0.04 (8)^{bc}	0.79 ± 0.03 (8)^{c}	0.8 ± 0.01 (7)^{c}	0.41 ± 0.01 (8)^{a}	0.41 ± 0.02 (8)^{a}	0.42 ± 0.02 (7)^{a}	0.42 ± 0.02 (8)^{a}
3	BMI WK3	0.92 ± 0.05 (8)^{b}	0.89 ± 0.03 (8)^{b}	0.92 ± 0.06 (8)^{b}	0.95 ± 0.03 (7)^{b}	0.5 ± 0.03 (8)^{a}	0.46 ± 0.03 (8)^{a}	0.47 ± 0.03 (7)^{a}	0.5 ± 0.01 (8)^{a}
4	BMI WK4	1.06 ± 0.07 (8)^{b}	1.07 ± 0.05 (8)^{b}	1.14 ± 0.08 (8)^{b}	1.17 ± 0.03 (7)^{b}	0.53 ± 0.02 (8)^{a}	0.53 ± 0.02 (7)^{a}	0.54 ± 0.02 (7)^{a}	0.52 ± 0.04 (8)^{a}
5	BMI WK5	1.35 ± 0.04 (8)^{b}	1.4 ± 0.05 (8)^{b}	1.38 ± 0.07 (8)^{b}	1.35 ± 0.05 (7)^{b}	0.58 ± 0.04 (8)^{a}	0.52 ± 0.02 (7)^{a}	0.59 ± 0.02 (7)^{a}	0.59 ± 0.01 (8)^{a}
6	BMI WK6	1.46 ± 0.19 (8)^{b}	1.53 ± 0.05 (8)^{b}	1.47 ± 0.1 (8)^{b}	1.45 ± 0.04 (7)^{b}	0.62 ± 0.02 (8)^{a}	0.65 ± 0.04 (7)^{a}	0.62 ± 0.03 (7)^{a}	0.64 ± 0.02 (8)^{a}
7	BMI WK7	1.3 ± 0.03 (8)^{b}	1.54 ± 0.06 (8)^{c}	1.45 ± 0.1 (8)^{c}	1.53 ± 0.05 (7)^{c}	0.67 ± 0.03 (8)^{a}	0.73 ± 0.03 (7)^{a}	0.7 ± 0.03 (7)^{a}	0.72 ± 0.02 (8)^{a}

TABLE 5: EFFECTS OF GENOTYPE (MARSHALL, HARCO) , DIETARY CABBAGE LEVEL (0,3,6 AND 12%) FROM 0-4 WEEKS AGE, AND THEIR INTERACTION ON BLOOD CHOLESTEROL.

Breed irrespective of Diet			**Diet Irrespective of Breed**				
Trait	**Marshall ALL**	**Harco ALL**	**0% ALL**	**3% ALL**	**6% ALL**	**12% ALL**	**Breed x Diet**
TC	278.85 ± 5.22 (20)*	151.37 ± 2.01 (20)	227.39 ± 28.66 (10)^{b}	213.1 ± 21.56 (10)^{a}	208.61 ± 18.52 (10)^{a}	211.34 ± 16.9 (10)^{a}	***
LDL	75.07 ± 1.63 (20)*	43.51 ± 1.58 (20)	59.42 ± 8.09 (10)	57.67 ± 5.98 (10)	59.8 ± 4.42 (10)	60.27 ± 3.34 (10)	***
HDL	140.57 ± 4.33 (20)*	70.35 ± 1 (20)	122.11 ± 16.43 (10)^{b}	105.84 ± 11.14 (10)^{a}	98.16 ± 9.73 (10)^{a}	95.73 ± 9.76 (10)^{a}	***

*group mean, standard error and count are presented as; means ± sem (n)

a, b ,c ,d means with different superscripts within row are significantly different.

TABLE 6: BLOOD CHOLESTEROL OF MARSHALL AND HARCO CHICKENS FED GRADED LEVELS OF CABBAGE (*Brassica oleracea*) FROM HATCH TO 4 WEEKS AGE.

	Marshall				**Harco**			
Trait	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**
TC	312.85 ± 6.56 (5)^{e}	277.23 ± 5.08 (5)^{d}	263.61 ± 4.14 (5)^{c}	261.71 ± 3.3 (5)^{c}	141.93 ± 0.52 (5)^{a}	148.97 ± 3.09 (5)^{ab}	153.61 ± 3.62 (5)^{ab}	160.97 ± 2.41 (5)^{b}
LDL	83.42 ± 2 (5)^{e}	75.28 ± 1.89 (5)^{d}	72.59 ± 2.19 (5)^{cd}	68.98 ± 3.12 (5)^{c}	35.43 ± 1.6 (5)^{a}	40.05 ± 1.47 (5)^{a}	47 ± 1.2 (5)^{b}	51.55 ± 1.61 (5)^{b}
HDL	171.24 ± 2.16 (5)^{e}	139.11 ± 1.67 (5)^{d}	127.1 ± 1.8 (5)^{c}	124.81 ± 1.59 (5)^{c}	72.97 ± 1.71 (5)^{b}	72.58 ± 1.52 (5)^{b}	69.21 ± 1.89 (5)^{ab}	66.65 ± 1.9 (5)^{a}

*group mean, standard error and count are presented as; means ± sem (n)

a, b ,c ,d means with different superscripts within row are significantly different.

Serum Cholesterol

Genotype factor effect on Serum Cholesterol: The broiler (Marshall) genotype had significantly higher Serum Total Cholesterol, LDL-C and HDL-C than the pullet at week 4 of the experiment (Table 5).

Diet (graded levels of Cabbage) factor effect on Serum Cholesterol: When all birds were considered irrespective of genotype, Serum Total Cholesterol, decreased with dietary administration of cabbage (0% Cabbage > 3%, 6%, 12% Cabbage) as shown in Table 5. A similar trend was observed for HDL-C (0% Cabbage > 3%, 6%, 12% Cabbage) as shown in Table 5. In contrast, dietary Cabbage had no significant ($p>0.05$) effect on LDL-C (Table 5).

Genotype x Diet (graded levels of Cabbage) factor interaction effect on Serum Cholesterol: Genotype significantly interacted with dietary Cabbage to determine Total Cholesterol, LDL-C and HDL-C at age 4 weeks (Table 5).

Serum Cholesterol effects of Graded levels of Cabbage within genotype (breed): In the Marshall Broiler, an inverse relationship was observed between dietary Cabbage level and Serum Total cholesterol (0% Cabbage > 3% Cabbage > 6% Cabbage, 12% Cabbage) as shown in Table 6. A similar trend was observed for HDL-C in the same Genotype (0% Cabbage > 3% Cabbage > 6% Cabbage, 12% Cabbage). Dietary Cabbage also significantly ($p<0.05$) lowered LDL-C, though the trend was more gradual (0% Cabbage > 3% Cabbage > 12% Cabbage, and 6% Cabbage intermediate between but not different from the 3% and 12% Cabbage groups) than observed for TC and HDL-C as shown in Table 6.

The pullet (Harco) genotype exhibited a direct relationship trend between dietary Cabbage and Serum Total Cholesterol levels, (0% Cabbage < 12% Cabbage, and 3% Cabbage and 6% Cabbage intermediate between but not different from either group (Table 6). A direct relationship trend was also observed between dietary cabbage and LDL-C in the Harco Pullet (0% Cabbage, 3% Cabbage < 6% Cabbage, 12% Cabbage) as shown in Table 6. In contrast, an inverse relationship was observed between dietary Cabbage and HDL-C (0% Cabbage, 3% Cabbage > 12% Cabbage, and 6% Cabbage intermediate between but not different from either group) as shown in Table 6.

Haematological parameters

Genotype factor effect on haematological parameters: Genotype significantly ($p<0.05$) determined haematology, and broiler (Marshall) genotype produced significantly higher White Blood Cell (WBC) Count, and Neutrophil, Basophil and Eosinophil differential WBC levels, whereas pullet genotype produced significantly higher PCV, Hb, RBC, Lymphocyte and Monophil levels (Table 7).

Diet (graded levels of Cabbage) factor effect on haematological parameters: When all birds were considered irrespective of genotype, dietary cabbage had no significant ($p>0.05$) effect on any of the haematological parameters examined in the present study (Table 7).

Genotype x Diet (graded levels of Cabbage) factor interaction effect on haematological parameters: Genotype did not significantly ($p>0.05$) interact with dietary Cabbage to determine any of the haematological parameters examined in the present study (Table 7).

Haematological effects of Graded levels of Cabbage within genotype (breed): In the Marshall Broiler, only the proportion of white blood cells represented by eosinophils was significantly determined by Diet (0% Cabbage > 6% and 12% Cabbage, and 3% Cabbage intermediate between but not different from either group) as shown in Table 8. In the Harco Pullet, none of the haematological parameters examined in the present study differed between dietary groups (Table 8).

TABLE 7: EFFECTS OF GENOTYPE (MARSHALL, HARCO), DIETARY CABBAGE LEVEL (0, 3, 6 AND 12%) FROM 0-4 WEEKS AGE, AND THEIR INTERACTION ON BLOOD HAEMATOLOGY GIRTH.

	Breed irrespective of Diet		**Diet Irrespective of Breed**				
Trait	**Marshall ALL**	**Harco ALL**	**0% ALL**	**3% ALL**	**6% ALL**	**12% ALL**	**Breed x Diet**
Pack cell volume (%)	25.67 ± 1.63 (21)	26.95 ± 1.37 (20) *	23.9 ± 2.71 (10)	25.9 ± 2.01 (10)	28.45 ± 1.46 (11)	26.7 ± 2.31 (10)	NS
Heamoglobin (g/dl)	8.53 ± 0.54 (21)	8.95 ± 0.45 (20)*	7.94 ± 0.9 (10)	8.59 ± 0.67 (10)	9.46 ± 0.47 (11)	8.88 ± 0.77 (10)	NS
White blood cell(10^3/ml)	10.73 ± 0.66 (21)*	9.52 ± 0.56 (20)	9.69 ± 0.71 (10)	10.12 ± 0.91 (10)	9.95 ± 0.77 (11)	10.81 ± 1.19 (10)	NS
Red blood cell (10^3/ml)	6.38 ± 0.35 (21)	6.74 ± 0.44 (20)*	6.49 ± 0.72 (10)	6.97 ± 0.53 (10)	6.08 ± 0.44 (11)	6.71 ± 0.56 (10)	NS
Neutrophil (%)	54.8 ± 1.18 (15)*	54 ± 0.78 (19)	54.3 ± 1.5 (10)	54.13 ± 1.38 (8)	54.78 ± 1.22 (9)	54.14 ± 1.45 (7)	NS
Lymphocyte (%)	43 ± 1.05 (15)	43.89 ± 0.77 (19)*	43.1 ± 1.34 (10)	43.63 ± 1.32 (8)	43.44 ± 1.12 (9)	44 ± 1.43 (7)	NS
Monocyte (%)	1.4 ± 0.27 (15)	1.58 ± 0.23 (19)*	1.8 ± 0.29 (10)	1.5 ± 0.33 (8)	1.33 ± 0.41 (9)	1.29 ± 0.42 (7)	NS
Basophil (%)	0.6 ± 0.13 (15)*	0.42 ± 0.12 (19)	0.5 ± 0.17 (10)	0.63 ± 0.18 (8)	0.33 ± 0.17 (9)	0.57 ± 0.2 (7)	NS
Eosinophil (%)	0.27 ± 0.12 (15)*	0.05 ± 0.05 (19)	0.3 ± 0.15 (10)	0.13 ± 0.13 (8)	0 ± 0 (9)	0.14 ± 0.14 (7)	NS

*group mean, standard error and count are presented as; means ± sem (n)

a, b ,c ,d means with different superscripts within row are significantly different.

TABLE 8: BLOOD HAEMATOLOGY OF MARSHALL AND HARCO CHICKENS FED GRADED LEVELS OF CABBAGE (*Brassica oleracea*) FROM HATCH TO 4 WEEKS AGE.

	Marshall				**Harco**			
Trait	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**	**0% Cabbage**	**3% Cabbage**	**6% Cabbage**	**12% Cabbage**
Pack cell volume (%)	22 ± 4.23 (5)	28.4 ± 1.83 (5)	28.17 ± 2.59 (6)	23.6 ± 4.08 (5)	25.8 ± 3.64 (5)	23.4 ± 3.43 (5)	28.8 ± 1.28 (5)	29.8 ± 1.59 (5)
Heamoglobin (g/dl)	7.3 ± 1.42 (5)	9.38 ± 0.66 (5)	9.38 ± 0.82 (6)	7.9 ± 1.36 (5)	8.58 ± 1.21 (5)	7.8 ± 1.13 (5)	9.56 ± 0.44 (5)	9.86 ± 0.56 (5)
White blood cell (10^6/ml)	8.94 ± 0.4 (5)	11.04 ± 1.37 (5)	10.88 ± 1.28 (6)	12.04 ± 1.88 (5)	10.44 ± 1.35 (5)	9.2 ± 1.19 (5)	8.84 ± 0.46 (5)	9.58 ± 1.46 (5)
Red blood cell (10^9/ml)	5.64 ± 0.52 (5)	6.76 ± 0.72 (5)	6.3 ± 0.57 (6)	6.82 ± 1.05 (5)	7.34 ± 1.3 (5)	7.18 ± 0.86 (5)	5.82 ± 0.74 (5)	6.6 ± 0.56 (5)
Neutrophil (%)	54.4 ± 2.5 (5)	53.75 ± 2.43 (4)	56 ± 2.48 (4)	55.5 ± 2.5 (2)	54.2 ± 1.96 (5)	54.5 ± 1.71 (4)	53.8 ± 1.07 (5)	53.6 ± 1.89 (5)
Lymphocyte (%)	42.4 ± 2.2 (5)	43.75 ± 2.32 (4)	42.75 ± 2.21 (4)	43.5 ± 1.5 (2)	43.8 ± 1.71 (5)	43.5 ± 1.66 (4)	44 ± 1.18 (5)	44.2 ± 2.01 (5)
Monocyte (%)	1.8 ± 0.49 (5)	1.75 ± 0.63 (4)	1 ± 0.41 (4)	0.5 ± 0.5 (2)	1.8 ± 0.37 (5)	1.25 ± 0.25 (4)	1.6 ± 0.68 (5)	1.6 ± 0.51 (5)
Basophil (%)	0.8 ± 0.2 (5)	0.75 ± 0.25 (4)	0.25 ± 0.25 (4)	0.5 ± 0.5 (2)	0.2 ± 0.2 (5)	0.5 ± 0.29 (4)	0.4 ± 0.24 (5)	0.6 ± 0.24 (5)
Eosinophil (%)	0.6 ± 0.24 (5)^{b}	0.25 ± 0.25 (4)^{ab}	0 ± 0 (4)^{a}	0 ± 0 (2)^{a}	0 ± 0 (5)^{a}	0 ± 0 (4)^{a}	0 ± 0 (5)^{a}	0.2 ± 0.2 (5)^{ab}

DISCUSSION

Effect of genotype on bodyweight irrespective of dietary Cabbage level: The results of the present study indicate differences in body weight of the two breeds examined irrespective of administration of cabbage (*Brassica oleracea*) in the diet, with the Broilers exhibiting significantly higher body weights compared to the Pullets, thus confirming the paradigm upon which the current study was based; that broilers grow significantly faster than pullets when fed the same diet as reported by Evaraert *et al*. (2008). These results are consistent with findings of Druyan (2010) who suggested that genetic selection for various traits caused different strains to have differing growth patterns, not only during embryonic development but also at post hatch stage. The genetic make-up of the respective breeds of chickens studied here was responsible for this distinction in body weight. Broiler chickens are raised specifically for their meat thus, over the years they have been genetically modified for fast growth (Havenstein *et al*. 1993), Pullets on the other hand are raised purposely for egg production and in essence they have been genetically modified to efficiently utilize feed to produce larger numbers of eggs.

Effect of dietary Cabbage level on bodyweight irrespective of genotype: Results of the current study showing that dietary cabbage at the levels examined (3 – 12%) conferred neither benefit nor dis-benefit with respects to body weight in the first 4 weeks from hatch, indicate that Cabbage may be applied as a non-conventional dietary feedstuff within the diet of broilers at the levels used.

The data support the popular view that early nutrition has a lifelong effect on productivity because in the latter stages of the experiment during which dietary supplementation with Cabbage had been discontinued, significant differences were observed in body weight between broiler groups initially fed different supplemental levels of Cabbage. Specifically, early nutrition with Cabbage at 3% supplemental dietary levels promoted growth as reflected in birds fed 3% Cabbage which had significantly higher body weights than the 0% Cabbage group.

Bodyweight effect of interaction between dietary cabbage and genotype (breeds): Outcomes of the present study point to a universal (genotype independent) mechanism by which dietary Cabbage influences bodyweight since no genotype x diet interaction effect on bodyweight was observed.

Effect of dietary Cabbage on bodyweight within genotype (breeds): An absence of feedstuff treatment effect on body weight of chickens (broilers and pullets) between weeks 1 and 5 is not unusual, given that others (Tekeli *et al.,* 2006) have also demonstrated no effect of supplemental plant material (extracts of *Yucca schidigera, Oreganum vulgare, Thymus vulgaris, Syzygium aromaticum* and *Zingiber officinale*) on bodyweight of Broilers from hatch to six weeks age. In the latter stage of the current experiment, between weeks 6 and 8 (after cabbage inclusion was discontinued), inter-breed differences in response to cabbage administration, with the Broilers fed 3% and 12% exhibiting significantly higher body weight than other genotype-matched groups while no significant difference was observed between Pullet dietary groups, showed that the respond to cabbage is not universal, and that different genotype responds to the same early nutrition in different ways though this nutrigenetic factor effect fails to achieve significance when tested.

Effect of genotype on BMI irrespective of dietary Cabbage level: The significantly lower BMI observed for Pullets relative to Broilers in the present study reflects the fact that the former are bred for egg laying are not bred to lay down much fat as fatness and excessive weight gain are known to adversely hamper reproductive fitness. It may also be because Pullet chickens have less intensity of feed intake compared to Broiler. Researchers including Havenstein *et al.* (2003) and Druyan (2010) have reported extensively on the genetic improvement of Broiler and Layer chickens for enhanced growth rate and meat yield or intensified egg production, respectively.

Effect of dietary Cabbage level on BMI irrespective of genotype: The absence of a measurable difference in BMI between dietary groups fed graded levels of dietary supplemental Cabbage indicates that it may be fed to both genotypes without adverse effect of increasing fatness, but equally confers no benefit in reducing the fatness/BMI, thus supporting the utility of Cabbage as a BMI-neutral non-conventional feedstuff which may be included in the standard repertory of ingredients used in the manufacture of poultry feeds.

BMI effect of interaction between dietary cabbage and genotype (breeds): The absence of genotype x diet interaction effect on BMI is consistent with a uniform absence of effect on BMI across genotypes, and points to a universal (genotype independent) absence of mechanistic action on BMI in fast and slow growing chickens (broilers and pullets respectively).

Body Mass Index effect of Graded levels of Cabbage within genotype (breed): The reduction in BMI at 7 weeks resulting from early nutritional supplementation with Cabbage (3%, 12%, 6% > 0% Cabbage) points to a benefit in reducing obesity associated heart disease risk in broilers (Marshall breed). The absence of effect in the pullet suggests nutrigenomic differences between genotypes in adaptation to early nutritional supplementation with Cabbage.

Diet (graded levels of Cabbage) factor effect on Serum Cholesterol: The data point to a heart protective effect of cabbage through its Total Cholesterol lowering action in chickens, despite its effect in lowering the level of so-called good cholesterol (HDL-C) and raising the level of so called “bad Cholesterol” (LDL-C) both of which are fractions of TC. The reduction of cholesterol by cabbage corresponds to earlier reports where other natural plant materials have been used. For instance, Canogullari *et al.* (2010) reported that garlic powder and thyme successfully reduced cholesterol levels in birds.

Genotype x Diet (graded levels of Cabbage) factor interaction effect on Serum Cholesterol: A nutrigenetic effect of Cabbage on cholesterolaemia indicates that not all genotypes respond equally to the same cabbage supplement, and individualised (genotype-dependent) prescription is required in recommending use of cabbage as a supplement in the diet of poultry where cholesterol levels are important.

Serum Cholesterol effects of Graded levels of Cabbage within genotype (breed): Whereas cabbage was effective in lowering Cholesterolaemia in broilers at 3% dietary supplement levels, the best results were obtained at 12 % supplementation levels in the broiler, the latter yielding greater heart protection. Because dietary cabbage also lowered LDL-C and HDL-C in broilers, the effect of cabbage is consistent with an effect in lowering cholesterolemia without marked impact on the ratio of HDL-C:LDL-C. A nutrigenetic effect of cabbage is evident in its contrasting effect on Pullets: Whereas it lowers TC and LDL as it does in broilers, its effect on HDL-C is opposite. Cabbage actually increases the levels of Good Cholesterol while lowering the overall levels of Total Cholesterol and Bad Cholesterol in pullets. The exact basis of their genotype driven contrasting responses is not clear. These data however indicate that in the pullet, the heart-healthy effect of cabbage is mediated through an overall reduction in cholesterolaemia and also an improvement in the profile of cholesterol in blood.

Effect of dietary Cabbage level on Haematology irrespective of genotype: Scientific literature indicated that liver and kidney damage which is indicative of toxicity of treatment agents ultimately produces inflammation which is measurable through the haematology indices (Marrota *et al*. 2006). The current study revealed no effect of dietary cabbage on the haematological parameters as measured here, and this showed the data point on the safety of cabbage and its potential utility as a non-conventional feedstuff for poultry.

Haematological effect of interaction between dietary cabbage and genotype (breeds): The absence of genotype x diet interaction effect on Haematology is consistent with a uniform absence of effect on Haematology across genotypes, and points to a universal (genotype independent) absence of mechanistic action on Haematology in fast and slow growing chickens (broilers and pullets respectively), at least of the specific genotypes examined here.

Haematological effects of Graded levels of Cabbage within genotype (breed): The Cabbage (6% and 12%) induced reduction in Eosinophil proportion of white blood cells in broilers (relative to 0% Cabbage group) points to a very specific inflammation-lowering effect in the genotype. The absence of a Cabbage effect on the same parameter in the pullet genotype would explain the lack of overall effect of cabbage on this parameter when all birds were considered irrespective of diet. The absence of effect of a plant-based feed ingredient on haematology in broilers is not unusual. Rather, it is consistent with the findings of Agbede and Aletor (2003) who fed another natural plant material (*Leucaena leucocephala* seeds) to Broilers chickens at inclusion levels of 0%, 3%, 6%, 9% and 12% and saw no significant haematological effects. The same authors (Agbede and Aletor, 2003) also observed no significant perturbation of haematology when fish meal was replaced with leaf protein concentrate from Glyricidia in diets for Broilers chicks.

CONCLUSION

The results of this study showed that birds fed 3% cabbage produced significantly higher body weight after week 4 to support the view that early nutrition has a lifelong effect on productivity. Analysis of body mass index and ratio of girth length reveals that cabbage supplementation in diet does not confer any benefit on modulation of obesity (as measured by body mass index). Dietary cabbage significantly reduced total cholesterol levels and also LDL cholesterol while increasing HDL cholesterol level in Broilers only. Cabbage supplementation suppressed broiler eosinophil levels indicating effects on mediators of innate immune surveillance, but did not influence any other blood haematological parameter. Thus cabbage can be a useful resource in combating the problems associated with higher cholesterol levels in the blood and help in the prevention of hypercholesterolemia, obesity and metabolic syndrome.

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