EFFECT OF FEEDING AMARANTH (AMARANTHUS HYPOCHONDRIACUS) SEEDS ON BLOOD SERUM PARAMETERS AND BODY WEIGHTS IN LACTATING SAANEN GOATS

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Abstract: - This investigation was carried out to evaluate the effect of incorporating 10% and 15% non-heat treated (NHT) and 10% and 15% heat treated (HT) Amaranth (*Amaranthus hypochondriacus*) seed powder into concentrate feed mixture consisting of rice (*Oryza sativa*) bran/polish and milling by products of black gram (*Vigna mungo*) fed to lactating goats (*Capra hircus*), on their body weight and blood serum parameters. A randomized complete block design with four replicates was used to compare five treatment groups. Control diet consisted of natural grass together with rice polish and milling by products of black gram, whereas Amaranth seed powder in different ratios as mentioned above was added to four experimental rations. All diets were formulated to meet NRC standards for dairy goats. Body weight was recorded and blood samples of the animals were drawn at the beginning of the experiment before commencement of the feeding trial and after every 30 days for a period of 03 months, during the feeding trial. The mean total cholesterol (TC) concentrations in the control, 10% NHT, 15% NHT, 10% HT and 15% HT experimental animals were 113.7, 112.03, 110.75, 101.99 and 120.01 mg/dL and the mean high density lipo-protein cholesterol (HDLC) concentrations were 75.48, 77.39, 72.56, 70.88 and 76.55 mg/dL respectively, at the end of the experiment. In contrast the mean low density cholesterol (LDLC) concentrations were 33.2, 28.6, 30.3, 27.8 and 38.3 mg/DL respectively, at the end of the experimental period. The mean triacylglycerol (TAG) concentrations ranged from 26.29-49.57(mg/dL), whereas mean weights of the lactating goats ranged from 39.35-47.42kg respectively, between different treatment groups. Total serum cholesterol, high density lipo-protein cholesterol and low density lipo-protein cholesterol content of all goats increased with the time irrespective of the diet. According to results, the tested feed rations have no significant effect (P>0.05), on the body weights of the animals and the tested blood serum parameters, compared to the control. Results from this study may hamper some of the initial enthusiasm with respect to potent cholesterol lowering properties of Amaranth products, in ruminant animals.

Key-Words: - Amaranth seeds, Blood serum parameters, Body weights, Dairy goats.

1 Introduction

Milk is one of the highly nutritive and most precious natural foods and has been a basic component of the human diet since antiquity. Milk has always been an important component in the normal balanced diet comprised with high caloric value, protein, calcium, vitamins and other minerals. Therefore, milk production represents a major component in global food production. In addition, in the present trend towards the vegetarianism in Sri Lanka, India and various other countries due to religious, health and various other reasons, milk plays a major role in providing essential amino acids which are lacking in vegetable based diets especially for the growing children, pregnant mothers, sick and elderly people.

However, patients affected by hyperlipidemia/hypercholesterolemia are reluctant to consume milk due to the presence of fat and cholesterol. For such people, if milk is totally removed from the diets, it would deprive them of many essential nutrients. In scientific literature, it has been shown that low cholesterol eggs and meat can be obtained by supplementation of poultry diets with Amaranth (*Amaranthus spp.*) with promising results (Punita *et al*., 2000; Khiroug, 2001; Berger *et*

al., 2003; Kroliczewska et al., 2008) [27], [18] ,[4] [5], [20]. Scientific findings suggest that Amaranth protein has a metabolic effect on endogenous cholesterol metabolism (Mendonça et al., 2009) [23]. It was observed that Amaranth seeds and oil had cholesterol lowering properties in experimental animals (Qureshi et al., 1996; Berger et al., 2003) [28], [4] [5]. Besides the above, a group of medical scientists have studied the Amaranth oil in human trials, and recommended Amaranth oil as a functional food product for the prevention and treatment of cardio vascular diseases due to its cholesterol and triglyceride lowering properties (Martirosyan et al., 2007) [22] . In addition, it has been shown that feeding Amaranth is associated with reduction in serum lipids and oxidative stress in rabbits (Kabri et al., 2011) [15]. However, no research has been carried out with dairy animals by incorporating Amaranth seeds in their diets as a supplement, to study the blood serum parameters.

However, literature suggests the presence of heat labile anti-nutrients such as saponins, trypsin inhibitors and tannins in Amaranth seeds (Jacob, 2015; Andrasofszky et al., 1998) [14], [2], and further stated that heat treatment could destroy the heat labile anti-nutritive compounds in Amaranth grains.

Therefore, this study was carried out to investigate the effects of supplementing Amaranth seed powder (heat treated and non-heat treated) to the basal diets of lactating goats on their body weight, milk composition, milk cholesterol levels as well as blood serum parameters (Total Cholesterol (TC), high density lipoprotein (HDL) Cholesterol, low density lipoprotein Cholesterol (LDL) and triglycerides.

However, this paper totally, focuses on the effect of feeding Amaranth seeds as a supplement, on blood serum parameters and body weights of lactating goats.

2 Materials and Methods

Twenty lactating Saanen goats of 2 to 4 years aged were used for the study. On the basis of weight, age and the lactation number, experimental animals were divided into two blocks (10 animals for each block, 30.6 ± 3.5 kg and 50.8 ± 7.2 kg). Animals within a block were divided into two similar groups and animals within a group were assigned to five experimental rations in a randomized complete block design with two replicates and were confined into single cages (1.5×1.5 m^2/animal).

Experimental rations were, control (basal diet), basal diet supplemented with 10% non-heat treated

Amaranth seeds (T_1) (10% NHT), basal diet supplemented with 15% non-heat treated Amaranth seeds (T_2) (15% NHT), basal diet supplemented with 10% heat treated Amaranth seeds (T_3) (10% HT) and basal diet supplemented with 15% heat treated Amaranth seeds (T_4) (15% HT) (Table 1). Basal diet consisted of natural grass, rice bran, milling by product of black gram, mineral and vitamin mixture and yeast with different proportions. Seeds of Amaranthus hypochondriacus were used for this study. Before the commencement of the trial, all experimental diets were offered gradually to the experimental animals for a period of 3 weeks for the purpose of pre-conditionsing of animals. All diets were formulated to meet NRC standards for dairy goats so that diets of 30 kg animals had an energy content of 1.99 Mcal/d and 100 g of digestible crude protein per day whereas diets of 50 kg animals had an energy content of 2.65 Mcal/d and 103 g of digestible crude protein per day (NRC, 2012) [24]. All heat treated and non-heat treated Amaranth seeds were ground (Bartkowiak et al., 2007) [3] by using a grinding machine (DIETZ WRB 80 C) and sieved to pass through a 1 mm screen. Amaranth seeds were heat treated by keeping the seeds in a preheated oven at 60^0C for a period of one hour to inactivate heat labile anti-nutritive compounds. Amaranth seeds were ground to prevent undigested seeds to pass through the digestive tract of the animal. All diets were mixed daily and daily allowance of the feed for all animals provided at once, just after morning milking. The weights of the refused feed were recorded daily.

Clean drinking water was available for the animals ad libitum. Body weights of the animals was measured by a floor weighing scale, at the beginning of the experiment before commencement of the feeding trial and after every 30 days for a period of 03 months.

Blood samples (4mL) were drawn at the beginning of the experiment before commencement of the feeding trial and after every 30 days for blood serum parameter estimation. Blood samples (10 mL from each animal) were drawn into clean vacutainer tubes from the animals in the morning before feeding, from the jugular vein and were allowed to clot for 1 hour at room temperature. After clotting, blood samples were centrifuged at 3000 rpm/min for 15 min. (Abdelhamid, 2012; Khan et al., 2013) [1] [16]and, subsequently the clear non haemolysed supernatant fresh serum was separated and stored at -20 ^{0}C until analysis (Talavera et al., 1984; Khalifa et al., 2013) [30] [19].

Concentration of TC, HDLC and TAG were determined in serum using Randox (Randox

Laboratories, Crumlin, United Kingdom) kits. LDL: HDL ratios were also calculated.

2.1 Total Cholesterol Estimation:

Total cholesterol was estimated by using the glucose oxidase/peroxidase enzyme kit (Randox,UK), by mixing 10 µl of serum with 1 ml of the enzyme mono-reagent and measuring the absorbance at 500 nm.

2.2 High Density Lipoprotein-Cholesterol Estimation

The very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were precipitated by the addition of equal amount of polyethylene glycol-6000 (200 g/L, in Glycine/NaOH buffer pH 10.0) to serum. After centrifugation, the supernatant which contained the high density lipoprotein fraction (HDL) was assayed for HDL cholesterol using the enzyme kit (Randox, UK). One ml of the reagent was reacted with 50 µl of the supernatant.

2.3 Estimation of triacylglycerol concentration:

The triacylglycerol concentration was estimated by glycerol phosphate oxidase/peroxidase enzyme kit (Randox, UK), using 10 µl of serum with 1 ml of the enzyme mono-reagent and measured the absorbance at 500 nm. Lipases present in the reagent hydrolyzed triacylglycerol to glycerol and free fatty acids and, the glycerol in the presence of ATP and glycerol kinase was converted to glycerol-3-phosphate. Quality control sera normal and high (Randox, UK), were used each time the serum lipid analysis was carried out.

2.4 Calculation of LDL- Cholesterol concentration

LDLC content was estimated using Friedewald's formula (Khan, 2014) [17].

LDLC (mg/dL) = TC (mg/dL) − HDLC (mg/dL) − TG (mg/dL)/5

2.5 Data Analysis

Data were statistically analyzed using General Linear Models Procedures (GLM) of SAS (2009) [29], and significance was declared at $P<0.05$.

Table 1: Composition of diets used in the study (Dry Matter basis)

Item	30 kg animal group					50kg animal group				
	Control	10%NHT	15% NHT	10%HT	15%HT	Control	10% NHT	15%NHT	10%HT	15%HT
Ingredients(g/day)										
Natural forage	450	450	450	450	450	840	840	840	840	840
Rice bran (Oryza sativa)	220	160	140	250	230	450	430	390	450	450
Milling by products of black gram (Phaseolus mungo)	580	560	540	470	450	550	470	460	450	400
Amaranth seeds (non-heat treated)	-	80	120	-	-	-	100	150	-	-
Amaranth seeds (heat treated)	-	-	-	80	120	-	-	-	100	150
Mineral and vitamin mixture	15	15	15	15	15	15	15	15	15	15
Yeast	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

NHT-Non Heat Treated   HT-Heat Treated

Table 2: Chemical composition of feed ingredients used in the study

Feed Ingredient	DM%	Ash%	Crude Protein%	Crude Fibre%	Ether Extract%	Ca%	P%
Natural Forage (30kg group)	15.0	11.4	16.9	25.1	2.2	0.45	0.31
Natural Forage (50kg group)	21.0	10.7	10.7	21.1	2.7	0.77	0.17
Amaranth (NHT)	89.3	4.8	17.2	9.6	5.7	0.49	0.53
Amaranth (HT)	90.6	9.3	15.7	11.2	4.6	0.77	0.17
Rice polish	82.7	7.7	12.3	8.1	4.5	0.09	1.13
Milling by products of Black Gram	89.8	3.5	18.05	24.5	2.3	0.48	0.21
Yeast	95.2	5.8	45.1	0.1	0.8	31.6	7.8

HT- Heat Treated   NHT-Not Heat Treated

Table 3: Average botanical Composition of diets used in the study (Fresh weight basis)

Feed Ingredient	Natural Forage 30kg group (%)	Natural Forage 50kg group(%)
Guinea 'A' grass (Panicum maximum)	37	40
Glyricidia (Glyricidia sepium)	14	28
Beru-Diyanilla (Ludwigia peruviana)	6	-
CO-3 (Pennisetum purpureum X Pennisetum americarnum)	3	6
Jak (Artocarus heterophyllus)	22	13
Wal Sooriyakantha (Tithonia diversifolia)	6	5
Other	12	8

3   Results and Discussion

Data was analyzed using covariance analysis, where initial blood serum parameters of animals were considered as the covariates. According to the results, feeding of lactating goats with a feed mixtures supplemented by different levels of Amaranth grain (different treatment levels), did not significantly influence the blood serum parameters studied in this experiment (P>0.05). However, effect of blocking and the covariate effect were significant (P<0.05) for total serum cholesterol and HDL cholesterol. Due to this reason, a separate GLM procedure was carried out for TC and HDL to find out the effect of blocking and the effect of covariate. Ultimately it was revealed that, initial value of cholesterol of experimental units has no significant effect on final cholesterol value of experimental animals (P>0.05). For the final values of triacylglycerol and LDL cholesterol, effect of the covariate and the blocking effect were not statistically significant (P>0.05).

Subsequently, data were analyzed by one-way ANCOVA by considering the initial weights of the animals as covariates. According to the results of the analysis, feeding animals in the different experimental groups, with different feed mixtures supplemented by different levels and differently heat treated Amaranth grain powder (different treatment levels), did not significantly influence (P>0.05) the blood serum parameters studied in the experiment. Effect of the covariate (initial weight of the animals) was statistically significant (P<0.05) for the final values of HDL and total cholesterol concentrations. However, covariate effect was not significant (P>0.05) for final LDL cholesterol and triacylglycerol concentrations.

The effect of supplementation of Amaranth seed powder on blood serum parameters of the lactating goats are presented in Table 4. According to the Table 4, inclusion of Amaranth seeds in the diet of goats did not have any significant (P>0.05) effect on total cholesterol HDL, and LDL content of blood compared to the control. Inclusion of NHT or HT Amaranth seeds did not have any significant effect on the serum LDL cholesterol content of lactating goats (Table 4). The lowest LDL cholesterol was recorded in goats fed with 10% HT seed diet and the highest value was recorded in 15% HT seed diet.

Several hypotheses have been proposed to explain one of the most cited abilities of Amaranth, which is the modulation of serum cholesterol levels of animals and humans by feeding Amaranth. Such hypotheses cited the content of unsaturated fatty acids (UFA) (Chavez-Jauregui *et al*., 2000) [9], amount of total and soluble fibre in Amaranth (Mendonca *et al.,* 2009) [23], amino acid profile of Amaranth protein (Berger *et al.,* 2003) [4] [5] are responsible for the modulation of serum cholesterol content of experimental units. In addition, the presence of phytochemicals as tocotrienols (Lehman, 1996) [21], phytosterols (Martirosyan *et al.*, 2007) [22], tocopherols and squalene (Martirosyan *et al*., 2007) [22] have also been proposed projecting a complex scenario.

As Danz and Lupton (1992) [11] reported that Amaranth consumption by rat had a cholesterol lowering effect in the serum, Chaturvedi *et al*. (1993) [8] reported the hypocholesterolemic effect of an Amaranth diet was able to reduce total cholesterol by 50%, experimental rats compared to the control diet. Those authors hypothesized that the unsaturated fatty acid content of Amaranth could have promoted the observed effect.

Qureshi *et al*. (1996) [28], however studied the effect of supplementing the diet with Amaranth on cholesterol biosynthesis in chickens and observed the decreased total and low density lipoprotein cholesterol values and unchanged HDL cholesterol concentrations. This study suggested the observed results are due to the presence of some potent inhibitors of cholesterol synthesis, in non-ruminants. Among the diversity of attempts carried out to show the cholesterol lowering power of Amaranth, in-vitro assays and animal models like rats, hamsters, rabbits and chickens have been used, besides intervention trials in humans. However, no ruminant models have been studied with Amaranth diets so far.

On the basis of the results of the above mentioned studies, it can be hypothesised that non-significant cholesterol levels between different treatments obtained in the present study may be due to the hydrolytic action of ruminal bacteria especially on the comparatively high levels of unsaturated fatty acids, in Amaranth grain powder based diets, compared with the non-ruminant animal models. Further, it can be suggested on the basis of literature, that due to the action of hydrolysis of UFA in the rumen, cholesterol lowering effect of UFA (Chavez-Jauregui *et al*., 2000) [9], has been decreased or diminished. As the cholesterol lowering effect in the digestive systems of non-ruminant experimental models has been already observed, cholesterol lowering effect of Amaranth based diets could be seen in non- ruminant animals and humans.

Therefore, to get the cholesterol lowering power of Amaranth based diets to ruminant animal products, it is desirable to conduct research aimed at determining the effect of rumen protected/stable

Amaranth diets to minimize or protect the fat from microbial degradation.

Presently, it is believed that the increased LDL cholesterol concentration in blood is attributable to lauric C12:0, myristic C14:0, and palmitic C16:0 acids, whereas the other saturated fatty acids found in milk neutralise their effect as they increase HDL cholesterol concentration in blood (Parodi, 2009) [26].

In the current study, total amount of lauric, myristic and palmitic acids produced by the different treatment groups are ranging from 28.5-34.0% compared to the 27.2% in the control diet, while the total amount of other saturated fatty acids ranging from 22.6-24.0% compared to the 27.2% in the control diet from the total amount of fatty acids in goat milk fat.

The study carried out by Nudda et al. (2013) [25] detected, 34.1% as the sum of lauric, myristic and palmitic acids in the control diet and 26.1% in the treatment diet, from the total fatty acids. The sum of other saturated fatty acids in goat milk fat in the same study was 25.4%, in the control diet and 28.9% (from the total fatty acids) in the treatment diet in the same study.

Inclusion of Amaranth seeds in the goat diet did not influence (P>0.05) the serum Triacylglycerol concentration (Table 4). However rate of increase of Triacylglycerol concentration of serum was very much higher for goats fed with 10% NH seed diet compared to other diets. Inclusion of 10% HT seed diet and the control diet reduced the rate of increase of serum Triacylglycerol concentration compared to other treatments. These results are similar to the opinion indicated by Bernard et al. (2005) [6] that the lack of significant variation of serum triglycerides of goats by diets differing in type and level of fat.

The present findings are in agreement with Castro et al. (2013) [7] and Berger et al. (2003) [4] [5], who reported from their studies that Amaranth oil and Amaranth flakes did not have potent hypocholesteromic effect in hamsters. Furthermore, according to Berger et al. (2003) [4] [5] particular Amaranth species and cultivars under specific growing and processing conditions, may have cholesterol lowering properties. However, A. hypochondriacus used in this trial does not show potent cholesterol lowering properties.

Table 4: Effect of different diets on blood serum parameters of lactating goats‡

Parameter	Treatment						
	Control	10%NHT	15%NHT	10%HT	15%HT	SEM	P value
TC (mg/dL)	113.7±43.6	112.0±47.9	110.7±35.2	101.9±23.6	120.0±28.	36.88	0.97
HDL-C (mg/dL)	75.4±19.2	77.4±30.4	72.5±19.6	70.9±19.1	76.8±22.3	22.5	0.99
LDL-C (mg/dL)	33.2±27.6	28.6±22.6	30.3±9.2	27.8±6.8	38.3±16.8	18.33	0.92
TAG(mg/dL)	30.3±11.4	40.1±18.6	49.6±33.1	26.3±5.7	34.8±7.4	18.24	0.44
LDL-C:HDL-C	0.4±0.2	0.4±0.2	0.4±0.03	0.4±0.1	0.5±0.3	0.20	0.85

‡Average of 4 animal's ± SD

TC - Total Cholesterol, HDL-C- High density Lipoprotein Cholesterol, LDL-C-Low density lipoprotein cholesterol, TAG-Triacylglycerol

Table 5: Effect of diet on Live weight gain of goats #, ##

Body weight	Treatments				
	Control	10%NHT	15%NHT	10%HT	15%HT
Live Weight (kg)	41±7.3	43.8±9.5	40.8±8.4	42.72±12.0	42.6±7.4
$(kg)^{0.75}$	16.2±2.1	17.0±2.7	16.1±2.5	16.6±3.5	16.6±2.0

NHT- Non Heat Treated,   HT- Heat Treated

[#] Average of 12 values of weight±SE

[##] Average of 24 values of dry matter intake ± SE

The body weights of the animals with the advancement of the age did not increase significantly (P>0.05) (Table 6). This finding is similar to the results obtained by Choi *et al*. (2004) [10] and Islam *et al.* (2002) [12] who observed that feeding with hydrogenated soybean oil containing about 21% conjugated linoleic acid, did not significantly affect the growth performance of rats. Furthermore, Islam *et al*. (2009) [13] observed similar results and reported that feeding palm oil and soybean oil did not significantly increase (P>0.05) the body weights of black Bengal goats.

4   Conclusions

Based on the results it could be concluded that the experimental feed rations have no significant effect on the tested blood serum parameters as well as body weights gain of the animals, during the experimental period. Further studies are needed to find out the effect of inclusion of high levels of Amaranth seeds on tested variables, to detect any deviations from the observed results.

To get the cholesterol lowering power of Amaranth based diets in ruminant animal products, it is appropriate to conduct research aimed to study the effect of rumen protected/stable Amaranth diets to protect the fat from microbial degradation.

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