Study on Milk Parameters of Saanen Goats Fed with Diet Containing Amaranth (*Amaranthus hypochondriacus*) Seeds

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Abstract: -This investigation was carried out to evaluate the effect of incorporation of 10% and 15% non- heat treated (NHT) and 10% and 15% heat treated (HT) Amaranth (*Amaranthus hypochondriacus)* seed powder to concentrate feed mixture consisting of rice (*Oryza sativa)* bran/polish and milling by products of black gram (*Vigna mungo*) fed to lactating Saanen goats *(Capra hircus)* on milk yield, composition, fatty acid profile and cholesterol content of milk produced by Saanen dairy goats over a period of 3 months.

A randomized complete block design with four replicates was used to compare five treatment groups. Control diet consisted of natural grass together with rice bran/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 Saanen dairy goats. Milk samples were collected before commencement of the trial and at every 2 week interval, after the commencement of the trial for a period of 3 months and analyzed for protein, fat, lactose and solid non-fat (SNF) using a Lacto scan milk analyzer standardized for goat milk. Milk fat extracted from the samples, by the modified Gerber method analyzed using a gas chromatography (GC) for fatty acid profile and cholesterol content, according to AOAC 996.06.

Addition of Amaranth seed powder into different treatment diets did not significantly influence the fat, protein, lactose and SNF contents in goat milk ($P>0.05$). Milk yields were also not affected by different treatment diets; 10% and 15% non- heat treated (NHT) and 10% and 15% heat treated (HT) Amaranth seed powder ($P>0.05$). Inclusion of Amaranth seed powder into different treatment diets did not cause a significant change in the fatty acid profile and the cholesterol content of milk fat compared with the control. However, there were significantly high ($P<0.05$) conjugated linoleic acid (CLA) concentrations in milk fat of all treatment groups, as well as in the control group.

Overall results indicate that, combination of feed ingredients in the diets, have favourable effects on milk fatty acid composition especially on CLA without negative effects on animal performance.

Key-words: - Amaranth seeds, Blood serum parameters, Body weight, Dairy goats.

1 Introduction

Amaranth (*Amaranthus spp.*) is a high yielding forage species with high nutritional value. Both the grain Amaranth and vegetable Amaranth has been utilized as a human food as well as forage for animals since the ancient times. Amaranth seed is an important grain and has a unique composition of protein, carbohydrates and lipids. In addition, amaranth grain also contains high levels of calcium, magnesium, iron, sodium and squaline, when compared to cereal grains (Berger *et al.*, 2003) [7] [8]. Amaranth oil consists of approximately 5 to 9% oil. Non-saturated fatty acid content of the oil fraction is 77% and from which predominant fatty

acid is linoleic acid (approximately 39-50%) (Jahaniaval *et al.*, 2000) [16].

For many years, research have been carried out to find out the effect of addition of specific type of plants into animal feed, consequently which can affect the health and productivity of the animal, as well as prophylactic and therapeutic properties of animal products, especially, nutritive value of animal products. According to literature, Amaranth (*Amaranthus hypochondriacus.*), possesses, these health supporting properties as well as ability to modify the composition of animal products, which is of great importance to human health (Bartkowiak *et al.*, 2007) [3].

Although several authors have examined the nutritive value of goat milk taking into consideration various factors (Matsushita *et al*., 2007; Pandya and Ghodke 2007; Strzałkowska *et al*., 2008) [24], [28], [37], Amaranth which is rich in linoleic acid has never been used to feed the dairy animals to improve the nutritive value of milk, to enrich the human diet with indispensable components.
Therefore, this study was carried out to investigate the effects of inclusion of Amaranth seed powder (heat treated and non-heat treated) to the basal diets of lactating goats on their milk yield, milk composition and milk fatty acid profile and the milk cholesterol levels as the information of the above mentioned areas are not available.

2 Materials and Methods

2.1 Geographical Location

This trial was carried out at a private goat farm at Gampola in the mid country wet zone of Sri Lanka (Mean annual temperature 24.6^0C; annual total precipitation 2689 mm; and the altitude 587 m).

2.2 Animals and Treatments

Twenty lactating Saanen dairy goats (*Capra hircus*) of 1 to 2 years aged at the beginning of the 1st and 2nd lactation were used in the first block, while lactating goats of 3-4 years aged at the beginning of 3rd and 4th lactation were used in the second block of the experiment in two different seasons (goats in the first block were treated in one season and goats in the second block were treated in another season). The experimental animals were stratified into two blocks (10 animals for each block, 30.6±3.5 kg and 50.8±7.2 kg), on the basis of body weight, age and the lactation number. 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 (2 reps/block * 2 blocks = 4 animals/treatment) and were confined into single cages (1.5x1.5 m^2/animal).
Experimental rations were, control, control supplemented with 10% non-heat treated Amaranth seeds (T_1) (10% NHT), control supplemented with 15% non-heat treated Amaranth seeds (T_2) (15% NHT), control supplemented with 10% heat treated Amaranth seeds (T_3) (10% HT) and control supplemented with 15% heat treated Amaranth seeds (T_4) (15% HT). Seeds of *Amaranthus hypochondriacus* were used in 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-conditioning of animals. All diets were formulated to meet NRC standards for Saanen dairy goats, so that diets of 30 kg animals had an metabolizable energy content of 1.99 Mcal/d and 100 g of digestible crude protein per day whereas diets of 50 kg animals had an metabolizable energy content of 2.65 Mcal/d and 103 g of digestible crude protein per day (NRC, 2012) [26]. All heat treated (Muyonga *et al.*, 2014) [25] and non-heat treated Amaranth seeds were ground (Bartkowiak *et al.*, 2007) [3] and sieved to pass a 1 mm screen. Heat treatment of the Amaranth seeds was performed by keeping the seeds in a preheated oven (60^0 C), for a period of one hour to inactivate, heat labile anti-nutritive compound. Amaranth seeds were ground to prevent undigested seeds to pass through the digestive tract of the animal.

2.3 Feeding Trial

All diets were mixed daily and daily allowance was provided at once, just after morning milking. The weights of the leftover feed were recorded next day morning. The samples of feed offered and refused feed were taken daily and dried at 60^0C for 72 h. Dry matter intake of the goats were calculated using the above information. All animals were clinically healthy during the investigation. Clean drinking water was available for the animals at all the time. Body weights of the animals were measured at the beginning of the experiment before commencement of the feeding trial and after every 30 days for a period of 03 months. The average of the initial and final weights was used as an estimate of metabolic size ($W^{0.75}$), based on which, intake of dry matter was calculated.

2.3.1 Sample Collection, Measurements, and Chemical Analysis

Determination of average botanical composition of the natural forage included in the experimental diets was done at two weeks intervals, by separation of each sample into different groups of same plants species and ultimately, weighing of the each group of samples.

At the end of the experimental period, dried feed samples and refused feed samples were composited and sub-samples were taken for chemical analysis. Sub-samples were ground by using a grinding machine (DIETZ WRB 80 C) to pass a 1 mm screen and subjected to proximate analysis (AOAC, 1990) [1].

2.4 Milk Sampling and Analysis

2.4.1 Milk Sampling

Milk samples were collected before the commencement of the trial and at every 2 weeks interval, for a period of 3 months. Animals were milked twice daily at 7.00 am and 3.00 pm and milk yields were recorded daily for each doe. The morning and evening milk samples were combined to get one sample (600 ml) from each animal on each sampling day.

2.4.2 Analysis of Milk Samples for Proximate Composition

Milk samples were analyzed for protein, fat, lactose and solid non-fat using a Milkco scan (TUV.CERT/Milcotronic, Bulgaria) standardized for goat milk.

2.4.3 Milk Fatty Acid Analysis

Milk fat was extracted from the samples (350ml) obtained from each animal by the modified Gerber method (Reaffirmed, 2003) [30] and stored at -20^{0}C until further analysis for fatty acid composition.

Fatty acid methyl esters (FAME) were prepared using milk fat samples already prepared and stored at -20^{0}C, and analyzed using a gas chromatograph (GC) equipped with a flame ionization detector (FID), according to modified AOAC 996.06. The individual FAMEs were expressed as relative weight percentage (AOAC, 2002) [2].

The GC-FID parameters (operation conditions maintained) were; carrier gas flow: Helium (1 ml/min, constant flow), Injection Volume: 1 µL, injection mode: split, Inlet heater temperature: 300^{0}C, Split ratio 50:1, detector: FID, heater on: 300^{0}C, Column type: HP 88. Oven program is; start 100^{0}C for 0 min, then 6^{0}C/min to 140^{0}C for 0 min, then 8^{0}C/min to 180^{0}C for 10 min, then 8^{0}C/min to 300^{0}C for 5 min, Run time 41.667 min, 5 min (post run) 300^0 C. Length of the column 60 m, diameter of the column 0.250 mm, particle size 0.20 µm.

2.4.4 Identification of FAME (Individual Fatty Acids)

The FAME were identified by comparison of the individual retention times of the sample constituents, according to fatty acid standards injected to GC with the sigma standards. The peak areas of FAME were determined with the software Agilent Mass hunter.

2.4.5 Quantification of FAME

Individual fatty acid were quantified and calculated as a percentage of sum of peak areas which was presented in chromatogram. Software used for calculation was Agilent mass hunter.

2.4.6 Milk Cholesterol Analysis

Milk fat samples extracted by modified Gerber method was used for cholesterol analysis. This method involved saponification of the sample with alcoholic potassium hydroxide and extraction of un-saponifiable matter in the sample, using n-hexane. Subsequently the extraction was, dissolved in 1 ml hexane and the solution was transferred into an auto sampler and injected to GC-FID system to determine the cholesterol content in the sample (internal standard 5α-cholestane was added). Elution of the internal standard (5α-cholestane) and cholesterol in the sample were noted. Their peak area was determined and concentration of cholesterol was calculated.

2.4.7 The GC-FID Parameters (Operation Conditions Maintained) for Milk Cholesterol Analysis

Carrier gas flow: Helium (1 ml/min, constant flow), injection mode: split, heater temperature: 300^{0}C, Pressure: 19.387 (psi), Septum purge flow: 3mL/min, Split ratio 50:1, Split flow: 53.99 l/Ml/min, initial oven temperature: 200^0 C for 1 min, then 20^{0}C/min to 300^{0}C, equilibrium time 0.1 min, maximum temperature: 350^{0}C, total run time: 16 min, Column type: J&W 122-1032 D.

2.4.8 Analysis of Fatty Acid Profile of Amaranth Seeds

Amaranth seed samples were ground by using a grinding machine (DIETZ WRB 80 C) to pass a 1 mm screen and fat was extracted using Soxhlet apparatus (AOAC, 1990) [1]

Fatty acid methyl esters (FAME) were prepared and analysed using extracted fat samples already prepared and stored at -20^{0}C, and analysed using a

gas chromatograph (GC) equipped with a flame ionization detector (FID), according to modified AOAC 996.06. The individual FAMEs were expressed as relative weight percentage (AOAC, 2002) [2].

The GC-FID parameters (operation conditions maintained) were; carrier gas flow: Helium (1 ml/min, constant flow), Injection Volume: 1 µL, injection mode: split, Inlet heater temperature: 300^0C, Split ratio 50:1, detector: FID, heater on: 300^0C, Column type: HP 88, Oven program is; start 100 ^{0}C for 0 min, then 6^0C/min to 140^0C for 0 min, then 8^0C/min to 180^0C for 10 min, then 8^0C/min to 300^0C for 5 min, Run time 41.667 min, 5 min (post run) 300^0 C. Length of the column 60 m, diameter of the column 0.250 mm, particle size 0.20 µm.

2.4.9 Statistical Analysis

Data were statistically analyzed using General Linear Models Procedures (GLM) of SAS (2009) [31] and significance was declared at $P<0.05$.

3 Results and Discussion

Cumulative results obtained for botanical composition of natural forge during the entire experimental period are given in Table 1. According to Table 1, forage was mainly consisted with Guinea 'A', a grass freely available in the area; Glyricidia, a fodder legume which is grown as a fence line and Jack, a tree used to cultivate to obtain edible fruits and, for timber. Table 2 shows the chemical composition of feed ingredients used in the study. Crude protein content of non-heat treated Amaranth was higher, whereas crude fibre content was lower when compared to heat-treated Amaranth. Milling by products of Black gram had the highest protein content compared to all the feed ingredients.

Higher levels of Conjugated Linoleic Acid-1(9C 11T) and lower levels of Conjugated Linoleic Acid-2 (10T-12C) were observed in non-heat treated Amaranth when compared to heat-treated Amaranth (Table 3). Composition of diets used in the study is given in Table 4.

Table 1: 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	0
CO-3 *(Pennisetum purpureum X Pennisetum amaricarnum)*	3	6
Jack (*Artocarus heterophyllus*)	22	13
Wal Sooriyakantha (*Tithonia diversifolia)*	6	5
Others	12	8

Table 2: Chemical composition of feed ingredients used in the study (%).

Feed Ingredient	DM	Ash	Crude protein	Crude Fiber	Ether Extract	Ca	P
Natural Forage (for 30kg group)	15.0	11.4	16.9	25.1	2.2	0.45	0.31
Natural Forage (for 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 product of Black gram	89.8	3.5	18.1	24.5	2.3	0.48	0.21
Yeast	95.2	5.8	45.1	0.1	0.8	31.6	7.8

Table 3: Fatty acid profile of Amaranth seed fat (g/100g of fat)*

Parameter	Non-heat treated	Heat treated
Caprilic Acid (C8:0)	0.1	0
Capric Acid (C10:0)	0.1	0
Lauric Acid (C12:0)	0.6	0.1
Myristic Acid (C14:0)	0.4	0.2
Palmitic Acid (C16:0)	12.6	15.2
Strearic Acid (C18:0)	0.5	0.5
Oleic Acid (C18:1)	13.9	14.5
Linoleic Acid (C18:2)	25.7	25.6
Conjugated Linoleic Acid-1(C18:2) (9C 11T)	17.6	11.2
Conjugated Linoleic Acid-2(C18:2) (10T 12C)	6.6	7.6
Linolenic Acid (C18:3)	0.1	0.1
Total Unknown	21.8	25.0

*Average of 3 samples

Table 4: Composition of diets used in the study (Dry Matter basis)

Item	30kg 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 (*Oryza sativa*) polish	220	160	140	250	230	450	430	390	450	450
Milling by product of black gram (*Phaseolus mungo)*	580	560	540	470	450	550	470	460	450	400
Amaranth Seeds (non-heat treated)	0	80	120	0	0	0	100	150	0	0
Amaranth seed (heat-treated)	0	0	0	80	120	0	0	0	100	150
Minerals 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

*Fresh weight basis

3.1 Feed Intake

Inclusion of Amaranth seed powder into diets, both as non-heat treated or heat treated form, did not affect (P>0.05) the daily DMI of experimental animals (Table 5). However, when the dry matter intake was calculated as a % of live weight basis or per metabolic body weight basis ($W^{0.75}$), the highest DMI was observed in 15% NHT diet whereas the lowest in 15% HT diet. Dry matter intake of HT diets was lower compared to other 3 diets and this may be due to some adverse effects of HT seeds. In general, dry matter intake as a % of live weight is 2-3% for ruminants and, the values reported in this study is ranging from 2.9 to 3.3%. According to INRA (2007) [15], DM intake per metabolic body weight is around 75g/kg $^{(0.75)}$ for small ruminants and the values reported in this study are in agreement with these reported values.

3.2 Milk Parameters

Table 6 presents the effect of different diets on milk composition and yield of lactating goats. According to the results, incorporation of Amaranth seed powder in different percentages in the experimental diets did not have any significant (P > 0.05) effect on the composition of milk or the yield. The lowest (P>0.05) milk yield was observed in the control diet, followed by 15% HT diet compared to 10% HT diet, which recorded the highest (P>0.05) milk yield. Milk yield reported for 15% HT diet was half of the value reported for 10% HT diet. In general, high milk yield is associated with a low % of milk and vice versa. However this relationship was not observed in milk when 15% HT was fed to animals.

Table 5: Effect of diet on dry matter intake (DMI) of goats [#, ##]

Dry matter intake	Treatments				
	Control	10%NHT	15%NHT	10%HT	15%HT
g/d	1312.2±367	1370.7±313	1348.3±320	1308.4±401	1240.2±521
g/(kg)$^{0.75}$	79.9±12.2	80.9±13.9	83.2±11.4	77.5±10.3	72.6±26.1

NHT-Non Heat Treated HT-Heat Treated

DMI- Dry Matter Intake

[#] Average of 12 values weight±SE

[##] Average of 24 values Dry Matter Intake ± SE

Table 6: Effect of different diets on milk composition and milk yield of lactating goats [‡]

Variable	Treatments						
	Control	10%NHT	15%NHT	10%HT	15%HT	SEM	P value (Treatment)
Fat%	6.19±2.3	5.2±1.3	6.1±1.2	6.3±1.3	5.7±0.7	1.6	0.7
Protein %	3.2±0.2	3.3±0.1	3.1±0.2	3.2±0.3	3.2±0.3	0.21	0.8
Lactose%	4.9±0.2	4.9±0.3	4.8±0.4	4.8±0.4	4.8±0.4	0.35	0.9
SNF%	8.8±0.4	8.8±0.1	8.8±0.6	8.8±0.7	8.9±0.3	0.45	0.9
Milk yield (l/d)	0.2±0.3	0.5±0.2	0.5±0.2	0.5±0.2	0.3±0.3	0.19	0.4

[‡] Average of 4 goats ±SD
(Average of 4 goats X 6 (2/month X 3 months) X 2 (twice)/day =48 samples

Table 7: Effect of different diets on Fatty acid composition and the cholesterol content of milk of lactating goats (g/100g of fat) %)

Variable	Treatments						
	Control	10%NHT	15%NH	10%HT	15%HT	SEM	P value
SFA							
Butyric Acid (C 4:0)	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.2	0.1	0.8
MFA							
Caproic Acid (C 6:0)	0.7±0.4	0.9 ±0.2	0.7±0.1	0.6± 0.4	0.9 ±0.2	0.2	0.7
Caprilic Acid (C8:0)	1.1±0.5	1.4±0.3	1.1±0.3	1.0±0.6	1.3±0.3	0.4	0.8
Capric Acid (C 10:0)	3.9 ±1.5	5.3 ±0.5	4.0 ±1.5	3.7 ±2.1	4.6±0.9	1.4	0.5
Lauric Acid (C12:0)	3.5±1.9	4.0±1.2	3.3±2.3	3.3±2.5	3.8±1.4	1.3	0.7
LFA							
Myristic Acid (C14:0)	6.1±2.1	9.1±2.3	6.3±3.5	6.8±3.6	7.9±2.3	1.4	0.2
Palmitioleic Acid (16:1)	0.5±0.2	0.5±0.4	0.5±0.3	0.6±0.2	0.4±0.3	0.2	0.5
Palmitic Acid (C16:0)	18.6±3.8	20.9±0.8	18.9±3.2	19.6±2.1	20.3±1.7	2.5	0.7
Strearic Acid (C 18:0)	15.7±3.9	16.1±0.7	17.1±2.8	17.0±4.1	15.6±0.9	2.9	0.9
Oleic/Vaccenic Acid (C18:1)	12.1±3.5	10.5±3.8	10.6±2.1	12.2±4.7	12.2±3.0	3.4	0.9
Linoleic Acid (C18:2)	8.3±2.3	6.0±0.7	10.0±5.1	7.6±2.8	6.3±1.3	2.0	0.3
Linolenic Acid (C18:3)	0.25±0.1	0.3±0.2	0.3±0.1	0.4±0.3	0.3±0.1	0.2	0.7
CLA1(C 18:2) (9C 11T)	6.3±3.7	4.2±1.4	5.7±2.7	5.9±2.6	5.0±2.5	2.8	0.8
CLA2 (C 18:2) (10T 12C)	0.8±0.4	0.6±0.3	0.6±0.2	0.9±0.6	1.6±0.7	0.8	0.4
Total CLA (CLA 1+ CLA 2)	7.2±3.7	4.8±1.2	6.3±2.6	7.0±2.3	5.9±2.5	2.7	0.7
P-S ratio	0.3±0.2	0.2±0.1	0.4±0.2	0.3±0.1	0.2±0.1	2.4	0.9
Milk Cholesterol	95.5±27.7	89.0±7.2	94.0±13.1	90.2±6.3	87.5±9.4	13.6	0.9

CLA (Conjugated Linoleic Acid), SFA-Short Chain Fatty Acid, MFA – Medium Chain Fatty Acid, LFA- (Long Chain Fatty Acid)

Table 7 reports the composition of the milk fat in terms of fatty acids and cholesterol. Inclusion of Amaranth seed powder into different treatment diets did not cause a change/significant modification ($P>0.05$) in the fatty acid profile and the cholesterol content of milk fat. However inclusion of HT Amaranth seed powder in the treatment diets markedly increased the CLA concentration in milk fat with non-significant differences ($P>0.05$) between treatments (Table 7). High levels of CLA in milk fat of experimental animals may be due to the high levels of CLA, and linoleic acid in HT Amaranth seeds (Table 3). As Chouinard *et al.* (2001) [9] stated, a portion of the high levels of CLA in fat supplements in animal feed, escape the bio-hydrogenation in the rumen and absorbed to the milk fat. Therefore, high levels of CLA in milk fat can be expected by escaping bio-hydrogenation in the rumen of the animal. On the other hand Amaranth seed contains 50% poly unsaturated fatty acids (Petras *et al.*, 2013) [29] in its fat and which contribute noticeably to the endogenous synthesis of CLA. Furthermore, Chouinard *et al.* (2001) [9] stated that with the ruminal bio-hydrogenation of polyunsaturated fatty acids in fat supplements in animal feed, trans-11 $C_{18:1}$ one of the intermediate formed and that escapes complete ruminal bio-hydrogenation.

The study carried out by Kubelcova *et al*. (2013) [21] revealed that addition of heat treated Amaranth seeds into the diets of the ruminants enhanced the production of microbial protein in the rumen effluent and in addition, no changes have been observed in concentrations of saturated and or unsaturated fatty acids. The last finding is in agreement with the results of the present study.
However it could be suggested that there may be a potential genetic effect in animals for the variables studied, rather than treatment effect on chemical composition and fatty acid profile of milk especially for the concentration of CLA in milk fat. Milk samples studied in the present study are containing greater concentration of CLA compared to the CLA values obtained in previous studies. The study carried out by D' Urso *et al*. (2008) [12] reported the CLA contents of 0.778% and 0.513% with pasture grazing and in house feeding with alfalfa hay with goats. The CLA values in the present study are much higher than these values (between 4.8% and 7.15%) reported by D' Urso *et al*., 2008 [12]. The high values of CLA in the present study might be due to the effects of high levels of CLA and linoleic acid in Amaranth seed powder (Table 03). In addition, high levels of CLA in all the treatment groups including control diet (although non-significant between treatments) may be due to the effect of natural forage, high levels of total unsaturated fatty acids in Black gram (82.9%) (Krishna *et al.* (1997) [20] and Rice polish oil (75%) (Wikipedia (2017) [40]. According to Chouinard *et al*. (1999) [10], the CLA in ruminant milk fat is depends on the bio-hydrogenation of polyunsaturated fatty acids by ruminal microorganisms and originates from two sources namely, a portion after escaping complete bio-hydrogenation in the rumen and, from endogenous synthesis of CLA in body tissues.

No significant differences were observed in the total concentration of polyunsaturated fatty acids (PUFA) as well as saturated fatty acids and the ratio between poly unsaturated and saturated fatty acids (P:S ratio) in milk between different treatment groups.

As Doreau *et al*. (1997) [11] stated, dietary lipid supplements composed of plant oils can produce inhibitory effects on microbial growth in the rumen. Beside this Bauman and Griinari (2000) [5] stated that use of plant oils as dietary lipid supplements alter the rumen environment so that a portion of the bio hydrogenation produces trans-10 $C_{18:1}$ and trans-10, cis-12 CLA, two metabolites associated with milk fat depression. In contrast, inclusion of Amaranth seed powder in to the experimental diets, milk fat depression was not observed in milk fat in the present study. This may be due to the reason that high levels of polyunsaturated fatty acids (Chouinard *et al.*, 2001) [9] in Amaranth seed powder which avoids undesirable effects on rumen bacteria.

In previous studies on the inclusion of lipids into ruminant diets as an energy source raised concerns about detrimental effects of fatty acids on ruminal fermentation (Jenkins, 1993) [17] . Lipids are extensively hydrolysed in the rumen, rendering fatty acids that have bacteriostatic and bactericidal effects. Among the hydrolysed lipids unsaturated fatty acids are more antimicrobial than saturated ones (Harfoot and Hazlewood, 1997) [14], and a different toxicity of different PUFA to rumen microorganisms has also been observed (Maia *et al.*, 2007) [22]. Dietary inclusion with oils has given inconsistent results on ruminal fermentation with detrimental effects (Fievez *et al*., 2003) [13], no effects (Keady and Mayne, 1999 [18]; Beauchemin *et al.*, 2007 [6]) and even positive responses (Sinclair *et al.*, 2005) [35]. Controversial outcomes may be due to the type (Wachira *et al*., 2000 [39];

Fievez *et al*., 2003 [13]) and amount (Shingfield *et al*., 2008) [34] of oil, but also to the basal diet composition.

On the basis of this, it can be suggested that very low milk fat content in 15% HT diet compared to all other treatments may be due to the detrimental effects of comparatively high poly-unsaturated fatty acids resulted from the hydrolysis of Amaranth lipids in the rumen of the lactating goats.

Controversial results may be due to not only to the type (Wachira *et al.*, 2000 [39]) of oil, but also to the basal diet composition and amount (Doreau and chillard, 1997; Shingfield *et al*., 2008) [11], [34].
According to Stoop *et al.* (2008) [36] there is a considerable genetic variation for fatty acid composition of milk and this variation is high for C4: 0 to C16:0 and moderate for c18 fatty acids. Results of the current research is comparable with this finding and there is a markedly different fatty acid profile of milk obtained from the present study, compared to the fatty acid profile presented by the other authors (Markiewicz-Keszycka *et al*., 2013) [23]. Further Stoop *et al.* (2008) [36] stated that fatty acid composition of ruminant milk can be changed by genetic selection of animals. Many authors have indicated that there is a significant association between genetic make-up and the milk fatty acid composition (Schennink *et al*. 2007; Bauman *et al*., 2011) [32] [4].

As Markiewicz-Keszycka *et al.* (2013) [23] further stated that it is difficult to enrich ruminant's milk with PUFA by changing the feed ration. However, as the authors further stated, advantageous variations of PUFA in cows, ewes and goats have been obtained by some authors by providing feed rations rich in green forages. Findings of the present study are in agreement with the authors and the resulting fatty acid profiles of the present study contains high levels of PUFA and CLA irrespective of the treatment effects. However, in contrast authors further stated that in most of the studies supplementing dairy cows, ewes and goats with vegetable oils or oil seeds have improved milk fatty acid composition with increased levels of beneficial nutritional factors (MUFA, n-3 PUFA).

Goat milk fatty acid profile presented by Markiewicz-Keszycka *et al*. (2013) [23], contains 3.0% PUFA while the milk samples obtained in the present study comprised with 6.3-10.3% PUFA two to three fold increase, irrespective of the treatment effect. Goat milk fatty acid profile presented by Same authors contains 0.45% CLA While the milk fatty acid profile of present study present study contains 4.8%-7.2% CLA, ten to 14 fold increase irrespective of the treatment effect.

Furthermore, Markiewicz-Keszycka *et al*. (2013) [23] stated that very desirable vaccenic acid content (which is a precursor of CLA in human organism) in ruminant milk is 1.5-5% of all fatty acids. However the vaccenic acid content in the present study (trial 01) is 9% in the control group and 6.2-11.2% (from all fatty acids) in the milk of the different treatment groups irrespective of the treatment effect, at the end of the experiment, which is a three to four fold increase compared to the natural ruminant milk.
The total saturated fatty acids (SFA) content detected in the control diet group of the present study was 49.8 wt. % and, in the treatment diet groups it was ranging from wt. 52.2-57.9%. However, these values were not significantly different ($P>0.05$).

The study carried by Nudda *et al.* (2013 [27]) found 62 wt% SFA values in the control diet group and 56.4 wt % SFA in the treatment diet group. Unlike the present study these values were significantly different ($P<0.01$).

Poly unsaturated fatty acid (PUFA) content detected in the control diet group of the present study was 15.7 wt% and it was ranging from 11.1-16.6% in the treatment groups. These values were not significantly different ($P>0.05$). However, Nudda *et al.* (2013) [27] observed significantly different ($P<0.01$), PUFA values of 6.2 wt % in the control group and 8 wt % in the treatment group.

These values clearly demonstrates that the milk produced by the experimental goats in the present study secrete more PUFA than the study carried out by Nudda *et al.* (2013) [27].

According to Markiewicz-Keszycka *et al*. (2013) [23], most abundant MUFA in ruminants milk is oleic acid (18:1) and it ranges between 20-35%. Several authors reported that oleic acid content (MUFA) in goat and sheep milk is on average 18% of all fatty acids (Schmidely *et al.,* 2011; Szumacher-Strabel *et al.,* 2011) [33] [38]. The oleic acid content goat milk fat of this study was 12.1% in the control diet group and 10.5-12.3% in the treatment groups. The Palmitioleic acid (16:1) content in ruminant milk was reported as relatively small (about 15%) in the previous reports (Markiewicz-Keszycka *et al*., 2013) [23], in

comparable to that the present study detected 0.5% palmitioleic acid in the control group and the 0.4%-0.6% in the treatment groups.

The total MUFA content in the present study was 12.6% in the control group and 10.9-12.9% in the treatment groups.

As Markiewicz-Keszycka *et al*. (2013) [23], stated that saturated fatty acids in ruminant milk account for 60-70% of fatty acids. However the total SFA content in the milk of the present study accounts for 50-60%. Markiewicz-Keszycka *et al*. (2013) [23], further stated that the main SFA in milk fat of the majority of the mammals is C16:0. Findings of the present study are in agreement with this statement and palmitic acid (C16:0) content in different treatment groups were 18.9-20.9% & the control group contained 18.6% Palmitic acid (C16:0).

3.3 Polyphenol Oxidase in Feed Ingredients and Fatty Acid Composition of Milk

At the outset of the present study, as reported by Kim *et al.* (2009) [19] it was assumed that the enzyme polyphenol oxidase (PPO) present in natural grass, that used to feed the experimental animals, would protect the poly unsaturated fatty acids in Amaranth seed powder, as well as other dietary lipids, from plant lipases after the ingestion of feed mixtures by the animal. According to literature, PPO is able to defend PUFA and retardation of lipolysis of ingested feed mixture in the digestive process of the animal. This would result increased delivery of PUFA from feed into milk. According to the observed results there has been a barrier to the above mentioned pathway, and altered the way of protecting lipids from lipolysis, by the PPO in natural grass and other feed ingredients.

The authors further stated that activation of enzyme PPO of forages would occur during mastication which provides a better opportunity for cell damage and aeration. The extent of activation of PPO is related to the stage of the maturity of the forage. If the forage has too much of fibre (high maturity), PPO content is low, in contrast too little fibre in immature forages results in very little mastication and rapid swallow with little cell damage and aeration to activate PPO. In the present study, maturity of the natural grass is unexpectedly uneven and cannot expect the proper levels of PPO to prevent dietary PUFA from lipolysis in the digestion process of the animal.

Furthermore, Kim *et al.* (2009) [19], stated that, activated PPO helps to reduce both lipolysis as well as bio-hydrogenation of dietary lipids in the rumen resulting in increased delivery of PUFA into animal products. The author further stated that, this is an important natural process to protect dietary lipids from bio-hydrogenation in the rumen.

In the present study Amaranth seed included into the feed, in the form of powder and therefore, it was observed that experimental animals swallowed the powder rapidly without mastication. Therefore, there were no room for the activation of PPO in Amaranth seed powder. In addition, there were lack of opportunity for mixing of forage with the feed mixture, and therefore, there were minimum opportunity for PPO in natural grass to be mixed together with Amaranth seed powder, and to minimise the retardation of lipolysis of UFA in it, in the digestive process of the animal.

Addition of Amaranth seed powder into the experimental diets does not appear useful with regard to the milk fat; protein, lactose, SNF, milk yields, and milk cholesterol are concerned. In contrary, very high levels of CLA in milk of all the treatment groups have also been observed in including milk of control animals.

According to the results of this study, it can be suggested that there may be a potential genetic effect of animals for the variables studied, rather than treatment effect on composition and fatty acid profile of milk, especially for the concentration of CLA in milk fat. In addition, it can be suggested that, natural grass, rice polish, milling by products of black gram may have a considerable contribution to the high levels of CLA in milk fat. High levels of CLA in Amaranth seed fat must have equally contributed, to the high levels of CLA in milk fat. Even though Amaranth seed fat has very high levels of CLA, cumulative effects of the other factors (genetics of animals, natural grass, rice polish, milling by products of black gram) may have masked the individual effect of Amaranth seed powder as Amaranth seed powder contains comparatively low level (5-6%) of fat compared with the contribution of other feed ingredients.

4 Conclusion

In conclusion, it can be said that incorporation of Amaranth seed powder into the diets of milking does, may not useful when the goats are fed on

comparatively high levels of natural forage, milling by products of black gram and rice polish. However, combinations of feed ingredients used in the diets of dairy goats for the present study, have favourable effects on milk fatty acid composition especially on CLA without negative effects on animal performance.

To get the desired fatty acid composition 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.

Furthermore, studies should be conducted to screen the genetic potential of the ruminant animals for high levels of CLA as well as favourable composition of fatty acids in products of the ruminant animals, in different farming systems of Sri Lanka.

In-vitro studies should be conducted to screen the micro-organisms responsible for the production of animal products with a favourable fatty acid composition, including CLA.

The decision for incorporation of Amaranth seeds to the diet of dairy goats should consider the productive efficiency of the animals especially the genetic effect of animals, as well as the associated changes in milk composition and the final value paid for the product in the market, by further investigations.

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