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Multi-Nutrient Milk Quality Analysis Applying Chemometrics: A Supplementation-based Approach using Dairy Goats

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ABSTRACT

Date pits (DP) are discarded as agricultural waste by-products and used in animals' supplementation. Data on multi-nutrient milk analysis is still less published to understand the effects of supplementation. Therefore, this research was done to evaluate the effect of DP powder (DPP) cultivars as supplementation on milk yield and quality to lactating Saanen-Boer crossed bred goats for a 12-week trial and to analyse the parameters using chemometrics. The analyses include milk yield, crude protein, fat, lactose and total phenolic content (TPC). The goats (n=24) were grouped into 12 designated cubicles and goats fed with normal daily rations, served as control. Several doses of DPP supplementations were administered against the control. Milk yield was significantly ($p < 0.05$) affected by DPP cultivars and doses. Significant ($p < 0.05$) increase in milk yield was registered for goats fed with A20 (59.52%) and M30 (28.24%), respectively compared to control. However, the crude protein (2.71 – 4.33%), fat (2.69 – 5.55%), lactose (4.52 – 9.66 mM) and TPC (0.14 – 0.42 mg/g) of the milk were not affected ($p > 0.05$) by the cultivar and dose. 3D PCA of the significant highest milk yield (A20 and M30) compared to the control focusing on combination of milk yield, crude protein, fat and TPC was obviously clustered. Hence, milk quality analyses via a multi-nutrient chemometric approach could be a comprehensive method in determining milk as food for security and sustainability.

1. Introduction

Various factors affect goat milk yield and quality. Among them are feed supplementation with other food resources which changes the macro-components (protein, fat and carbohydrate) and

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micro-components (small molecules) of milk as reported by [1] and many other researchers in the field of animal nutrition. Zenou and Miron [2] reported that ewes with fed soy hull pellets produced higher average milk yield (2.41 kg/d) compared to the control group (2.10 kg/d). In another study, lactating goats fed with 50 g/head/d of sunflower seeds whole (SS) or 20 mL/head/d of sunflower seeds oil (SO) improved milk yield and milk composition by enhancing the content of healthy fatty acids (conjugated linoleic acid and omega 3) without detrimental effects on the animal performance [3].

Approximately 20% of dates produced annually in the Arab peninsular are inedible and not suitable for human consumption [4]. Dates are commonly consumed as fresh fruit or turned into a variety of products such as confectionaries and date syrups, and in most cases the date pits or seeds end up as waste by-products of date fruit-processing plants [5]. These researchers noted that date pits contain significant but variable amounts of macro and micronutrients depending on the variety. All varieties however are excellent sources of dietary fibre (good quality), and may therefore serve as important constituents of functional foods, except for the poultry industry as highlighted by [6]. Other fruit seeds and agricultural waste byproducts that are utilized for different practical purpose include papaya seeds [7], honeydew melon rind [8] and Durian and Passion fruit peels [9].

Studies on the feasibility of date pits in animal feeds were first carried out by [10] for dairy cows. Furthermore, no toxicity is expected from seed extracts with previous studies reported no adverse effects of date pits on organ function, lipid profile, protein metabolism, haematological parameters and body weight of male Wistar rats [11]. AL-Suwaiegh [12] fed date pits with 0% (control), 10%, 15% and 20% to replace the concentrate feed to Ardi goats for 90 days, which did not significantly (p > 0.05) increase the milk yield. In contrast, average goat milk yield was highest in diet containing 1% of date palm seed meal (DPSM) compared to the control, as shown by [13].

Similarly, replacement of normal diet with 50% enzymatically treated date pit powder did not show a harmful effect on the goats [14]. Reference to Ajwa dates was made in “Hadith” and Islamic historical literature, because it is believed that eating this variety will cure many chronic ailments. The Ajwa date fruit is one of the most popular and expensive date palm fruits, fetching 3 times the price of the next best variety, and belongs to the holy city of Al-Madinah Al-Munawara and its adjoining areas in Saudi Arabia [15].

Goat breeds that are normally found in Malaysia include Katjang, Jamnapari, Boer and Savanna breeds [16]. They are mainly bred for their meat, while Saanen, Alpine, Toggenberg and Shami (Damascus) goats however are raised for milk production [17]. Despite that, the Anglo-Nubian and Jamnapari are among the dual-purposed (milk and meat) breeds that are imported. The possibility in looking at the goat as a potential source of protein (milk and meat) and livelihood to help feed and uplift these rural communities is of paramount importance [18]. Additionally, it is common for dairy farms in Malaysia to farm cross breeds between Saanen and Boer goats to improve the milk production [19].

Chemometrics can be used post instrumentation to reduce the dimensionality of the data and to examine the systematic variation in a large data matrix and thereby to identify the underlying variables that contribute to the differences between milk samples [20]. In a separate study, principal component analysis (PCA) of milk serum was able to differentiate between the control and restricted-fed groups, but not between breeds, which by default could mean that milk is a sample with more marked dissimilarities in contrast to other biofluids in goats when comparing to the mammary gland tissue [21].

It is hypothesized that by applying chemometrics on a multi-nutrient data, a different view or perspective of the DPP effects on milk from goats would facilitate in assessing the alteration that occurred. Furthermore, scientific publications on a multi-nutrient analyses and presented as

chemometrics view are still scarce. They focussed on multi-nutrient fortification of the milk outside the body, not as interventions given to the animals. Hence the aim of this study was to investigate the effect of Ajwa DPP (highly valued) and Mariami DPP (agricultural waste by-product) supplementation to Saanen-Boer crossed bred goats, on milk yield and quality (crude protein, crude fat, lactose content and TPC) over a duration of 12 weeks and to analyse simultaneously the multi-nutrient parameters using chemometrics.

2. Methodology

2.1 Ethical Approval

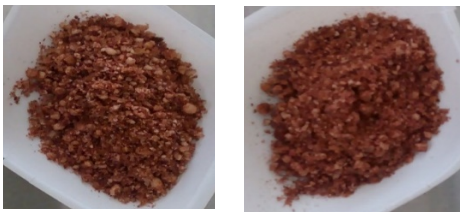
Animal studies were approved by the Animal Ethics Committee (AEC) of Universiti Sains Islam Malaysia [USIM / AEC / AUP / 2016 (3)].

2.2 Animals

The study was conducted at a private farm in Sg. Buloh, Selangor, Malaysia (GPS: 3.197592; 101.524848). Six groups of one year old Saanen-Boer cross female goats (n=4 per group) with a mean body weight of 24.89 ± 3.08 kg were used in the experiment. Prior to the study, the animals were subjected to a general health inspection by the farm owner and the veterinarian in the research team to ensure that the goats were in good condition. The goats were then acclimatised and fed with the basal diet (BD), composed of several food types as in section 2.3 prior to kidding. All goats were randomly divided into two goats per cubicle (6 x 5 feet), and natural impregnation was allowed over a month's period. Kids were born after 5 - 6 months gestation and allowed to suckle for the first week and according to specific time alloted throughout the study, after which they were separated from their mothers. The lactating goats were then managed appropriately to ensure that they were healthy throughout the study period.

2.3 Composition of Basal Diet (BD) and Date Pit Powder (DPP)

Each lactating goat was fed a BD comprising of approximately 500 g each of pellet, fresh Napier leaves and rice hay, respectively per day throughout the 12-week feeding trial (Table 1). DPP from two cultivars, Ajwa (highly-valued) and Mariami (by-product from the production of confectionaries) that were purchased from Syarikat Abdul Gaffar (SAG) in Penang, north of the Malaysian Peninsular (Fig. 1 a and b).



(a) (b)

Fig. 1. (a) Ground Ajwa DPP and (b) ground Mariami DPP

Table 1
Nutrient composition (%) of the BD used in the feeding trial

Feed type	Composition (%)				
	Dry matter	**Crude protein**	**Fat content**	**Crude fibre**	**Total ash**
Fresh Napier grass	15.18 ± 0.03	16.40 ± 1.34	1.97 ± 0.42	32.05 ± 0.68	12.26 ± 0.67
Grower pellet	90.57 ± 0.06	17.81 ± 0.88	5.15 ± 1.24	23.92 ± 1.72	7.90 ± 0.22
Rice hay	86.20 ± 0.12	6.19 ± 0.16	0.90 ± 0.24	37.38 ± 0.49	11.83 ± 0.20

Note: Data are presented as means of triplicates, Source: Reproduced with permission from [22]

2.4 Feeding Trial

Pellets from Nutri Vet Trading Company, fresh Napier leaves and rice hay were given to the goats separately at 08:00, 12:00 and 16:00 hours daily, respectively. The daily dose of 10 g of Ajwa DPP (A10), 20 g of Ajwa DPP (A20), 10 g of Mariami DPP (M10), 20 g of Mariami DPP (M20) and 30 g of Mariami DPP (M30) per goat per day was given to the goats by mixing with the pellets, while goats in the control group were fed with BD alone. At the end of the morning meal session, the tray was empty; all the DPP was ingested along with the pellet. The equivalent percentage of DPP in relation to the BD is shown in Table 2. Clean water was supplied by an automated dispenser *ad libitum*. The feeding trial began on the eighth day after lactation and lasted for 12 weeks.

2.5 Milk Sample Collection and Storage

The udder of the goats were wiped with a clean damped cloth prior to being hand-milked once daily into individual-labelled newly manufactured milk plastic bottles until the udder was reasonably empty at 14:00 by an experienced worker, supervised by the farm owner. The milk was then immediately stored in the refrigerator at 2-4°C. Subsequently, the kid was left to feed for the rest of the day [23] before being separated from its mother prior to morning meal (08:00) the next day until the milk sampling time (14:00) of the same day. Milk samples from the farm were brought in an ice-box to the laboratory twice a week, volume-recorded and were pooled at the laboratory after each visit, frozen in 50 mL Falcon bottles, freeze-dried and stored in powdered form at -20°C for further analyses.

Table 2
Feeding treatments comprising of the BD and DPP supplementations

Group	Treatments	BD + percentage (%) of DPP in diet
C	control (untreated)	BD only
A10	10 g of Ajwa DPP	BD + 0.67 DPP
A20	20 g of Ajwa DPP	BD + 1.33 DPP
M10	10 g of Mariami DPP	BD + 0.67 DPP
M20	20 g of Mariami DPP	BD + 1.33 DPP
M30	30 g of Mariami DPP	BD + 2 DPP

***Note*:** Control refers to goats which did not receive DPP supplementation. n=4; BD=basal diet; DPP=date pit powder

2.6 Determination of Nutrient Contents in Milk

The crude milk protein, fat, lactose and TPC contents for all treatments were determined following the procedures as described below. Analyses were done on the milk samples after the DPP treatment to the lactating goats as suggested by Martin *et al*., [24].

2.6.1 Protein

The crude protein content in goat milk was determined using Gerhardt VAPODEST 50 by the Kjedahl method [25]. Approximately 1 g of freeze-dried milk powder was used. One Kjeldahl tablet was mixed with 10 mL sulphuric acid (1 M) inside the digestion tube with the milk powder. The mixture was digested at 400°C for 30 mins and followed by distillation using dH_2O, NaOH and boric acid. The last step required the titration stage, using 0.1 N HCl and the pH-meter and was done automatically *via* the VAPODEST 50.

2.6.2 Lactose

Milk lactose content was determined using ^1H NMR spectroscopy as described by Klein *et al*.,[26].

2.6.3 Fat

Crude milk fat was determined following the method described by Bligh *et al*., [27] with modifications. Approximately 1 g of the freeze-dried milk sample was mixed with 60 mL chloroform: methanol (2:1) solvent and vigorously mixed using the homogenizer for 2 mins. Then the mixture was filtered using Whatman No. 1 filter paper using a Buchner flask. The mixture was then washed with the same solvent (40 mL) and transferred into a separating funnel. A 20 mL dH_2O was added and shaken before leaving it overnight for separation into two layers to occur. The lower layer was collected in a pre-weighed beaker and dried in the oven at 90°C for 4 h. Then the beaker with the dried lipid was transferred into the desiccator until it cooled and is weighed. Crude fat content was calculated as follows:

$$\% \text{ Crude fat} = \left[\frac{(\text{Weight of beaker with lipid} - \text{Weight of empty beaker})}{\text{Weight of sample}} \right] X\ 100 \qquad (1)$$

2.6.4 Total Phenolic Content (TPC)
2.6.4.1 Sample Preparation for Milk TPC Extraction

Extraction of TPC in milk was carried out according to the methods previously described by [28] with modifications. Solution of HCl (1 N) in 95% ethanol (v/v, 15/85) was used as the solvent for extraction of TPC from the milk powder. Ten millilitre (mL) of the solvent was added to 1.5 g of milk powder in 50 mL amber bottles and homogenized for 1 h at 30°C in a rotary shaker (Sastec) set at 300 rpm. The mixture of solvent and powder was then centrifuged at 7800 x G (Novil) at 5°C for 15 mins. The supernatant was collected and kept at -20°C in the dark until further analysis for TPC.

The TPC of freeze-dried goat milk powder was determined as described by [29] with modification. The supernatant (100 µL) was added to 0.4 mL distilled water and 0.5 mL of freshly

diluted 10-fold Folin-Ciocalteau reagent (Merck). After 5 min, 1.0 mL of 7.5% Na_2CO_3 solution was added to the mixture and allowed to react for 120 min at ambient temperature. The absorbance of the mixture was measured at 765 nm using an ELISA reader (Epoch, BioTek) against distilled water as blank. Gallic acid was used as the standard for the calibration curve at concentrations of 0.05, 0.10, 0.15, 0.25 and 0.50 mg/mL. TPC was expressed as mg gallic acid equivalents (GAE). All analyses were performed in triplicates.

2.6.4.2 Preparation of Gallic Acid Standard

A) ***Gallic acid stock solution***. A 0.1 g of powdered gallic acid was dissolved in 1 mL of ethanol and diluted to 10 mL using a volumetric flask with distilled water.

B) ***Sodium carbonate solution***. A 20 g of anhydrous sodium carbonate (Na_2CO_3) was dissolved in 80 mL of distilled water and made up to 100 mL with distilled water.

C) ***Preparation of calibration curve***. Each respective volume of 0.0, 0.1, 0.2, 0.3, 0.5 and 1.0 mL from the gallic acid stock solution in A) were added into individual 10 mL volumetric flasks and then diluted to the respective volumes with distilled water. These solutions had phenol concentrations of 0.00, 0.05, 0.10, 0.15, 0.25 and 0.50 mg/mL gallic acid respectively.

D) ***Absorbance reading***. Twenty microliter of each concentration solution (sample or blank), was mixed well with 1.58 mL distilled water and 100 µL of the Folin-Ciocalteu reagent. The solution was then left for 5 min prior to addition of a 300 µL Na_2CO_3 solution, and shaken to mix well before being left at 20°C for 2 h. The final solution was pipetted into individual wells of the ELISA plate reader and absorbance was determined for each sample at 765 nm against the blank using ELISA reader (Epoch, BioTek). Analyses were done in triplicates and a graph of absorbance *vs*. concentration was then constructed.

2.7 Statistical Analyses

The dependent variables were analyzed by using the SPSS statistical package software, IBM version 20 (SPSS Inc., Chicago, IL). According to the following model:

$$Y_{ab} = \mu + t_a + t_b + \varepsilon_{ab} \quad (2)$$

Y_{ab} is the trait studied (Milk yield, crude protein, fat, lactose or TPC); μ is the overall mean; t_a is the a^{th} DPP cultivar treatment (Ajwa or Mariami) including the control; t_b is the b^{th} DPP dose treatment (10, 20 or 30 grams) including 0 gram for the control; and ε_{ab} is the error term which is assumed to be randomly and normally distributed. Differences between treatment means were considered significant when the *p* values were less than 0.05 ($p < 0.05$) using ANOVA. Only three goat milk data (n=3) from each nutrient parameter (Crude protein, fat, lactose and TPC) were statistically analysed and presented. Chemometrics were applied using PCA and PLS (if appropriate) for potential cluster analyses (Unscrambler *X* software by Camo Software AS, Oslo, Norway). Data were mean-centred and scaled before the analysis.

3. Results and Discussion

3.1. Milk Yield and Quality from Goats Supplemented with DPP

The date pits used in this study were ground into powder to facilitate the mixing with feed pellet and to improve nutrient availability [12] as it was better tolerated and mimicked as the feed concentrates of the staple feed given to the goats. This current study utilised the Saanen-Boer crossed bred for potential milk yield increase and resistance towards potential local environmental intimidations for example climate change, pathogenic resistance and food acclimation. This concept of resistance to certain unwanted conditions were proven in bacteria [30], plants [31, 32] and animals [33].

Feeding the lactating goats with different doses of Ajwa or Mariami pits respectively, did not result in any significant ($p > 0.05$) changes in milk production throughout the study (Table 3). During this period, weekly milk yield fluctuated regardless of treatment and no significant ($p > 0.05$) differences were observed between the groups except during week 4. By week 10, yield per day for the A20 increased significantly (260.91 ± 98.17 mL) compared to all treatments except for M30 (164.82 ± 89.13 mL). Although yield per day for all treatments decreased by week 12.

The A20 treatment continued to give significantly ($p < 0.05$) higher yield (214.07 ± 62.08 mL) compared to the remaining treatments. This decrease in milk yield over time is consistent with possible loss of secretary cells after the peak lactation as shown by [34]. It is noteworthy that the milk yield weekly patterns in response to diet may differ between the goat breeds as reported by [9] who showed that supplementation with date pits to Ardi goats did not have any significant effect on the milk yield.

Statistical analysis showed no interaction between the date type as well as the feeding level with time on milk yield, except for week 6 for date type and time (Table 3). The cause for this occurrence is unclear. There might be other factors excluding breed and quality of the diet that pose an effect [18] on the milk yield. Furthermore, data on both milk yield and physical and chemical properties can provide information throughout a lactation period on the quality of milk [35].

Besides biological variability [36], several reasons could have attributed to the weekly fluctuations in the milk production observed in this study, one of which is the pattern of mammary gland alveolar development [37]. The goats used in the study were one year old and lactating for the first time; hence it is likely that differences in development of secretory cells in the mammary gland among the goats could have caused variations in the milk production. Indeed differences in udder morphology which became apparent only postpartum could also have contributed to the underlying results [38].

It was further noted that at week 2, M20 had a standard deviation greater than its mean milk yield value (122.75 ± 138.29 ml). This was due to milk yield ranged from 29 to 327 mL for the goats in M20 at that respective week. Jansen *et al*., [39] stated that when an intervention is implemented on several biological replicates, their response should be similar to the other replicates, up to a certain deviation caused by accepted and methodological differences. It is also highlighted by Chilliard *et al*., [40] that physiological status of the animal influences the inconsistency of milk yield and quality in goats and ruminants.

Environmental conditions such as temperature, humidity, solar radiation and wind speed which affect the animal thermoregulation [41], may also affect milk yield. During the study period (between March - May 2016), there was intermittent spells of rain fall and dry weather affecting the humidity and temperature. According to Salama *et al*., [41], milk yield in dairy goats decreases as temperature humidity index (THI) increases, and each unit increment of THI causes a 1% reduction in the milk yield. In addition, [21] also showed that in tropical and sub-tropical continents, goat milk yield varied

substantially throughout the year. In a separate observation by [42], data from dairy cows studies demonstrated that heat-stress (HS) during the dry period (i.e. last two months of gestation) reduced the mammary cell proliferation, and consequently decreased the milk yield in the subsequent lactation.

Table 3
Biweekly mean milk yield (ml – day^{-1}) from the Control and treated goats over the 12-week feeding period

	Treatments						Date type		Feeding level		Interaction	
W	Control	A10	A20	M10	M20	M30	A	M	10g	20g	Date type x Time	Feeding level x Time
2	130.29 ± 101.36	135.04 ± 59.63	213.76 ± 123.93	92.25 ± 57.09	122.75 ± 138.29	224.38 ± 67.45	174.40 ± 99.38	146.46 ± 104.05	113.65 ± 58.69	168.25 ± 130.94	NS	NS
4	106.25 ± 44.72^{b}	129.68 ± 59.01^{ab}	194.00 ± 57.38^{a}	136.75 ± 40.57^{ab}	100.00 ± 28.73^{b}	159.16 ± 68.75^{ab}	161.84 ± 63.92	131.97 ± 51.11	133.21 ± 47.03	147.00 ± 65.49	NS	NS
6	156.88 ± 101.73	168.21 ± 43.32	183.46 ± 37.57	145.25 ± 35.03	113.75 ± 53.20	166.15 ± 59.49	175.84 ± 38.41	141.72 ± 50.77	156.73 ± 38.48	148.61 ± 56.63	*	NS
8	130.43 ± 120.65	119.18 ± 28.32	183.68 ± 59.77	136.00 ± 17.11	131.00 ± 49.86	161.58 ± 65.68	151.43 ± 55.35	142.86 ± 46.15	127.59 ± 23.45	157.34 ± 58.22	NS	NS
10	126.75 ± 92.02^{b}	125.88 ± 62.87^{b}	260.91 ± 98.17^{a}	125.25 ± 29.57^{b}	107.50 ± 27.45^{b}	164.82 ± 89.13^{ab}	193.39 ± 105.04	132.52 ± 56.89	125.56 ± 45.48	184.21 ± 105.72	NS	NS
12	112.04 ± 61.25^{b}	113.36 ± 53.66^{b}	214.07 ± 62.08^{a}	119.00 ± 40.82^{b}	120.00 ± 39.27^{b}	112.14 ± 47.66^{b}	163.71 ± 76.05	117.05 ± 38.83	116.18 ± 44.24	167.03 ± 69.57	NS	NS

Note: Data with different superscripts in the same row and * indicate statistically significant ($p < 0.05$) differences by Duncan's test; n=4; W=Weeks; C=Control; A=Ajwa DPP; M=Mariami DPP; mean ± SD; NS=Not significant using independent samples t-test; x=interaction

Consistent with the trends in weekly milk yield, significantly ($p < 0.05$) highest mean total milk yield of 17.41 ± 6.17 L throughout the 12-week feeding trial was obtained from goats supplemented with A20 followed by the M30 treatment (13.99 ± 6.18 L). No significant ($p > 0.05$) differences in milk yield were obtained for the A10 (10.73 ± 3.61 L), M10 (10.63 ± 3.10 L), M20 (9.94 ± 4.28 L) groups and control (10.91± 7.44 L) (Fig. 2).

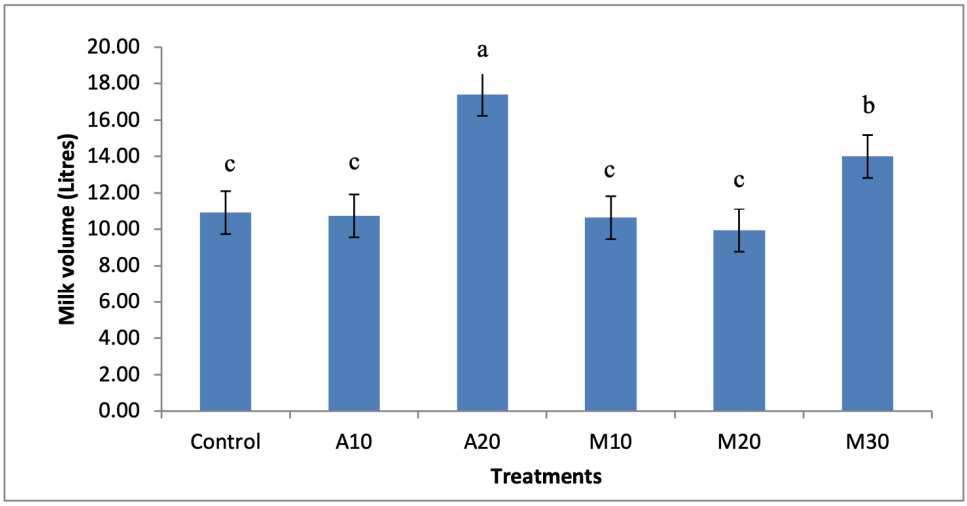


Fig. 2. Cumulative milk yield from goats supplemented with different doses of Ajwa and Mariami DPP for 12 Weeks. Treatments with different letters indicate significant ($p < 0.05$) differences (n=4, mean ± SD). A=Ajwa DPP; M=Mariami DPP

Further, monthly milk yield (Fig. 3) also showed a continuous increasing trend compared to the other groups up to the end of the trial for the A20 treatment. For the monthly milk yield changes in percent due to DPP supplementation as seen in Table 4, indicated that the highest percent increase was for A20 (95.38%) at month 3 of the trial and the lowest being at month 2 for M20 with 15.68%. All of which were compared to the control. No trend was identified for all the groups except for M30 with an exponential decrease from 45.06% to 18.91% at the end of the study.

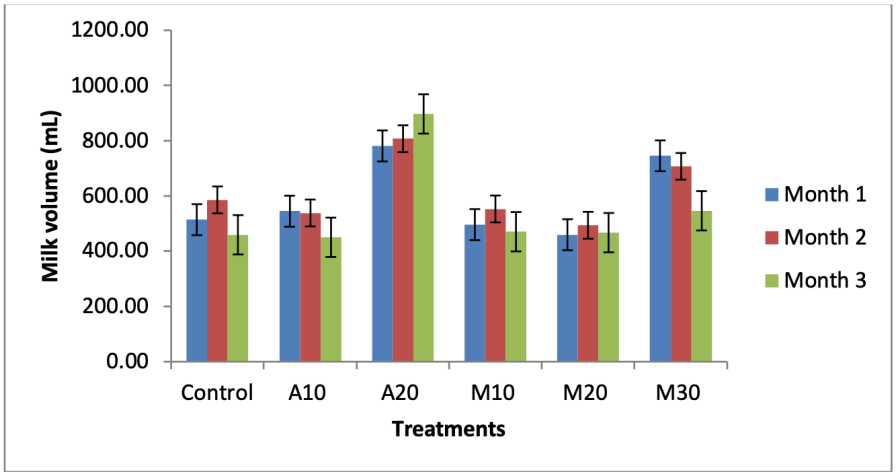


Fig. 3. Monthly mean goat milk yield (n=4, mean ± SD) from lactating goats supplemented with Ajwa or Mariami DPP at different doses. A10=10 g Ajwa DPP; A20=20 g Ajwa DPP; M10=10 g Mariami DPP; M20=20 g Mariami DPP; M30=30 g Mariami DPP

Table 4
Milk yield changes with time in percent (%)

Treatment groups	Week		
	4	8	12
A10	5.96	- 8.09	- 1.98
A20	52.09	37.91	95.38
M10	- 3.49	- 5.60	2.39
M20	- 10.59	- 15.68	1.68
M30	45.06	20.79	18.91

***Note*:** Milk percentage is compared to the Control at each respective month (n=4). (-) indicative of decreasing milk yield. A=Ajwa DPP; M=Mariami DPP

For the maintenance of milk quality however, it depended on the management of milk storage among others. Millogo *et al*., [43] concluded that raw milk can be stored at +4°C in the refrigerator during four days without any significant damage to the milk. In the case of this current study, milk was stored at the same temperature (+4°C) but for three days before further analyses. In contrast, Jaafar *et al*., [22] and Warly *et al*., [44] stated that their milk samples were stored at -20°C prior to further analyses.

No significant ($p > 0.05$) differences between the treatments were noted in the milk quality (crude protein, crude fat, TPC and lactose contents) throughout the trial duration with values ranging between 18.54 ± 6.37 to 29.60 ± 2.66 mg / g, 16.53 ± 6.55 to 37.92 ± 25.26 mg / g, 0.22 ± 0.01 to 0.41 ± 0.22 mg / g and 4.52 ± 0.48 to 9.66 ± 3.53 mM, respectively, (Figs. 4 to 7) suggesting that the type of DPP and dose used in the study did not influence these nutrients. This was in spite of the higher protein and fat intake of goats in the Ajwa DPP treatments as a consequence of the significant ($p < 0.05$) differences in the protein and fat contents of the Ajwa (6.35 ± 0.06 %; 6.12 ± 0.40 %) and

Mariami (5.48 ± 0.04 %; 4.10 ± 0.46 %) DPP in this study. According to [45], moisture, protein, oil and carbohydrate contents can vary from 3.1–12.5, 2.3–6.9, 5.0–12.5 and 70.9–86.9 g/100 g date–pits, respectively, depending on the date cultivar. Since the results presented in this study were from milk in freeze-dried form, values were much higher due to the greater chemical composition concentration over water ratio than reported by [22], except for the milk lactose content which was analysed from the raw milk.

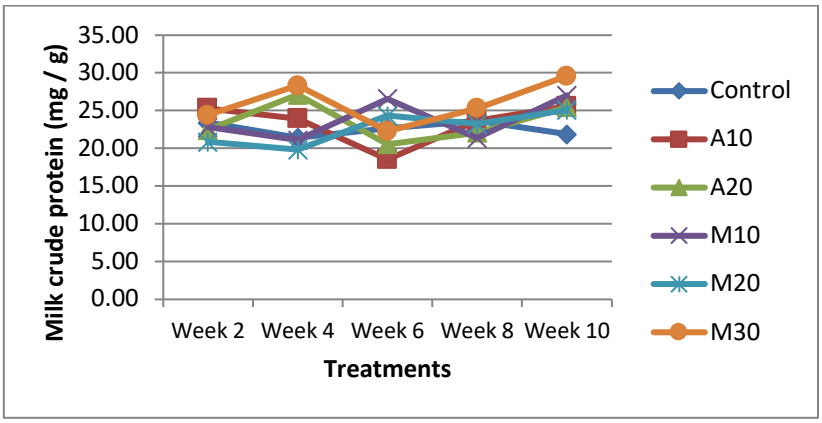


Fig. 4. Crude protein content (mg/g) of dry matter (n=3)

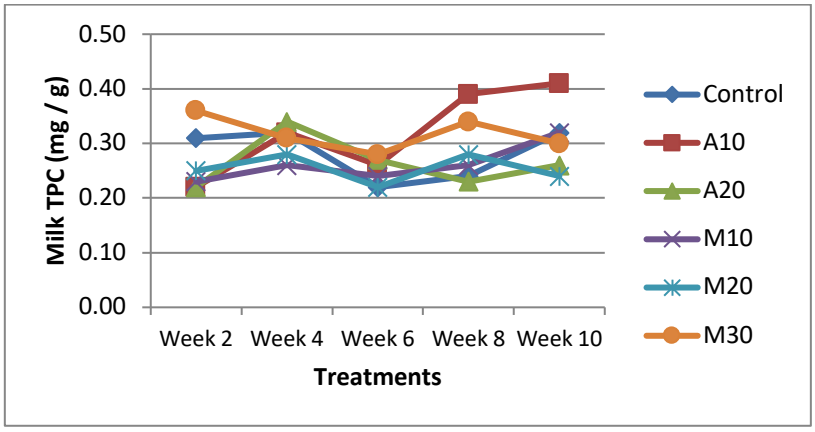


Fig. 5. Total phenolic content (mg /g) of dry matter (n=3)

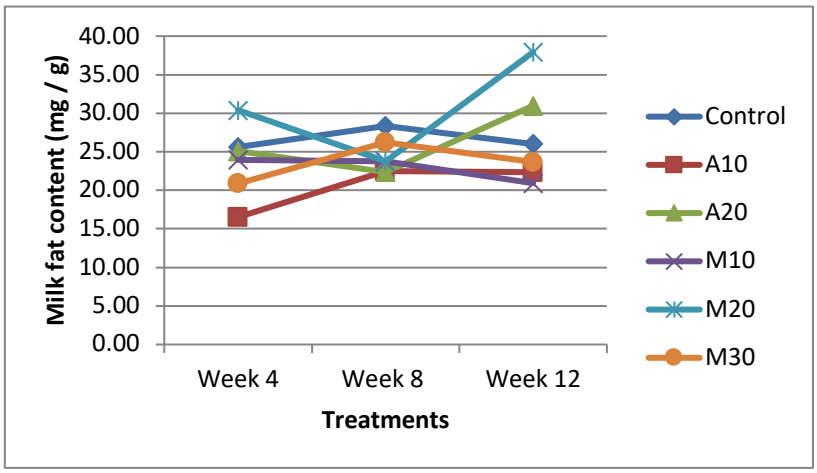


Fig. 6. Crude fat content (mg /g) of dry matter (n=3)

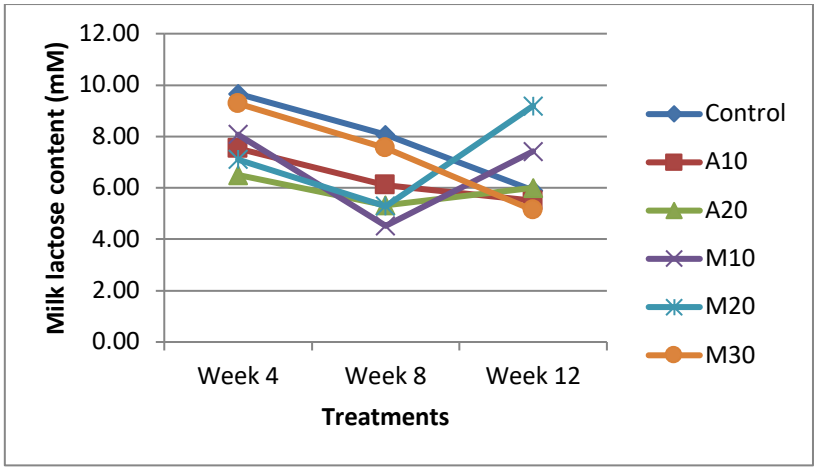


Fig. 7. Lactose content (mM) of wet basis (n=3)

Increased frequency of milking enhances milk yield and reduces secretory cell loss, whereas goats hemi-mastectomized at peak lactation undergo compensatory changes in the remaining gland, which include a complete maintenance of cell number for at least 18 weeks. Cell proliferation is increased in both cases, showing that mammary growth can occur during established lactation [34]. In this study, when a kid dies, milking has to continue to prevent mastitis or other complication to the goat mammary glands. When there is a kid to suckle the nipples, milk yield is higher during milk collection compared to the milk produced when the kid is not available. However, Gonzalo *et al*., [46] pointed out that udder health deteriorated in sheeps that suckled two lambs instead of one. In Targhee sheep, Gross *et al*., [47] found that the incidence of positive California mastitis test increased as the number of lambs born and weaned increased. These studies show that suckling influenced later udder health during the milking period.

Evidence on the influence of dietary protein and fat intake on milk yield and quality in goats and ruminants has been inconsistent, probably due to variations in breed [48], physiological status of the animal [40], experimental design [49] and DPP varieties and percent included in the diets [12]. For example, Al-Suwaiegh [12] reported a lack of significant differences in milk fat regardless of dietary fat intake. This was in contrast to [50], who reported that milk fat is more sensitive to dietary manipulation compared to protein or lactose. Further, the influence of energy balance with respect to the composition of the diet [51] as a key in determining milk crude protein and fat content just as milk composition and its relationship to the volume of milk produced [52], cannot be disregarded. In another perspective, accuracy of prediction of milk production (including protein and fat) is important for speeding up the identification of genetically superior animals, resulting in shorter generation intervals and thus in greater genetic progress [35].

The effect of diet on milk antioxidant capacity, based on total phenolic content (TPC), is well established [44]. Although Hamad *et al*., [53] showed that TPC in Ajwa date cultivars was highest [22.11 mg/100 g in dry weight (DW)] compared to 11 other date cultivars, no significant ($p > 0.05$) differences were detected in the Ajwa (1.69 mg/100 g DW) and Mariami (1.66 mg/100 g DW) DPP used in this study, and hence the similar milk TPC from the DPP-treated goats which ranged from 140 to 420 mg/100 g DW, regardless of treatment and feeding duration, was expected. The differences in the results could be due to the type of solvents used in the extraction of the phenolic compounds as solvent polarity influences the dissolution of compounds. Since there is increasing evidence that the antioxidant capacity of dates is closely associated with their TPC, the extracts with higher levels of TPC will exhibit greater antioxidant activities [54].

Additionally when referring to humans, reduction in total antioxidant capacity during the course of lactation and its relation to maternal antioxidant status needs more attention about the nutritional status. Many studies prefer to evaluate the *in vivo* efficacy of breast milks with different levels of total antioxidant capacity [55]. With time, the milk has a superior ability to scavenge free radicals from 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, inhibit their chain formation and destroy both initiation and propagation chains that lead to cancer and other diseases [56]. From a different perspective, [57] investigated the effect of hot season and nutrition on the oxidative status in dairy goats. The concentration of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and α-tocopherol was not affected by nutrition, but these factors were influenced by season. They concluded that in summer lactating goats may have experienced moderate oxidative stress. It seems that seasonal rather than nutritional factors have a more pronounced effect on oxidative status markers in dairy goats.

Another factor contributing to the antioxidative potential of the milk is the storage container where the milk is kept. Mestdagh *et al*., [58] compared the storage effects between glass and high-density polyethylene (HDPE) bottles for ultra-heat treatment (UHT) milk and [59] for low-fat pasteurized milk. Both of them found that there was no significant effect of packaging. Van Aardt *et al*., [60] detected higher oxidation off-flavour intensity in HDPE as compared to polyethylene terephtalate packaging and glass for full-cream pasteurized milk. However, the higher oxidation off-flavour intensity in HDPE is most likely caused by the shape of the container rather than of the oxygen permeability, since HDPE containers allowed for larger headspace volumes.

No significant ($p < 0.05$) differences in the overall milk lactose content was identified. Paralleled to the control, all treatments presented a decreasing trend except for the M10 and M20 treatment. Studies have revealed that lactose levels not only decreases as lactation period progresses and the decrease in milk production in contrast to fat, ash and total solids, which are susceptible to many factors as explained earlier [61,62], the content of milk lactose is also affected by the level of blood glucose in ruminant [63]. Indeed, lactose content is not influenced by diet except under extreme and unusual feeding situations [50]. Nevertheless the differences in composition of goat milk observed in this study compared to the other studies depend on several factors, the most important being the diet [64]. For example, Warly *et al*., [44] proved that feed supplement containing antioxidant of pineapple rind meal with Zn and Cu supplementation would reduce blood and milk cholesterol and increase milk lactose of goat milk. In addition, milk lactose concentration in this study was determined using ^{1}H NMR spectroscopy as shown by Monakhova *et al*., [65] and applying the manual identification method using Chenomx Profiler software as compared to the conventional enzymatic-based analysis [66].

Furthermore, a study by Hussein *et al*., [67] showed that chicks' supplemented with date pit diet had improvement in body weight and feed utilization. Aldhaheri *et al*., [68] found that using date pit as a part of the diet of Winstar rats gave no effect to the testosterone level of male rats, while the increase in date pit intake by the female Winstar rats caused the oestradiol in the rat's serum level to decrease. However, the results of the study showed a disagreement with Hussein *et al*., [67], where the addition of date pit into the Winstar rats diet had no effect on the rats total body weight gain. Another study carried out on Awassi lambs found that the additional of date's by products (date pits and flesh) in the diets increased the average daily gain, weight gain and back fat deposition of the lambs, which can be due to the presence of natural anabolic agents in date's by products [69]. Moreover, Habib *et al*., [11] reported that feeding 7 or 14% of date pit to rats ration increased malondialdehyde (MDA) content in both the serum and liver.

3.2 Chemometrics

3.2.1 Principal Component Analysis (PCA)

Since conventional analytical methods may not provide comprehensive information about the interactive effects of nutrients on milk quality and yield, a multivariate analysis (MVA) using PCA was applied to the data. PCA was performed to obtain an overview of the basic variation among the samples being analysed and to determine the presence of outliers [70]. The Scores plot of PCA analysis of crude protein, fat and TPC in milk as well as milk yield collected on days 30 (Month 1) and 60 (Month 2) and its Loadings plot are presented in Fig. 8. As the milk samples were of biological fluids and data used were of dissimilar parameters (milk yield, crude protein, fat and TPC), it was challenging to get a good clustering of the data. In spite of that, the results showed some possible clustering of the Ajwa and Mariami DPP treatments. The clustering between the Ajwa DPP group in relation to the control was more obvious as compared to the Mariami DPP groups and control, thus indicating a multivariate effect even though separate analyses of individual nutrients (protein, fat and TPC) in milk did not show any significant ($p > 0.05$) differences. Lactose values were not included in the PCA as lactose was quantified *via* whole milk, which included moisture content of the samples and thus would not give a true explanation of the result of the analyses.

Likewise, the same figure showed that PCA with the control and Mariami (M10, M20 and M30) DPP treatments were almost in the similar cluster. Variances were seen among the various Mariami DPP treatments. A comparison between Ajwa and Mariami DPP cultivars which have some impact on the PCA model, showed that DPP doses have lesser contribution to the model. The first 2 PCs generated by PCA described 74% of the total variances and that all the samples in the Scores plot were within the 95% Hotelling T2 ellipse. It can be observed that the separations of samples according to their treatments were seen on PC 2 as most of the Ajwa samples have positive scores whereas the control and majority of the Mariami samples have negative scores suggesting that the quality of milk is affected by the DPP treatment.

PCA was used to assess the overall level of the sample by describing the largest variance with the least number of components as described by Sun *et al*., [71]. In a Scores plot the data sets exhibiting similarities are clustered together, and those that are different are placed further apart [72]. Also in Fig. 8, differences among the A10 and A20 groups compared to the control were made visible. This clustering is possible due to the dissimilar DPP supplementation, as it was the main factor that was the same throughout the 12 weeks of the overall feeding trial suggesting that Ajwa DPP groups was contradictory in affecting milk yield and quality compared to the Mariami DPP groups. Although the tested variables were not having similar chemical characteristics, some clustering patterns were noticed especially in differentiating between the Ajwa and Mariami DPP-supplemented goats. This generally emphasizes the interactions among the nutrients which univariate analysis could not comprehend.

Furthermore, two dimensional (2D) Scores plot comparing the control and the significant ($p < 0.05$) highest milk-yielding groups (A20 and M30) did not show any clear clustering configurations, typical of biological fluids such as milk [73] due to natural progression with time and diet [74].

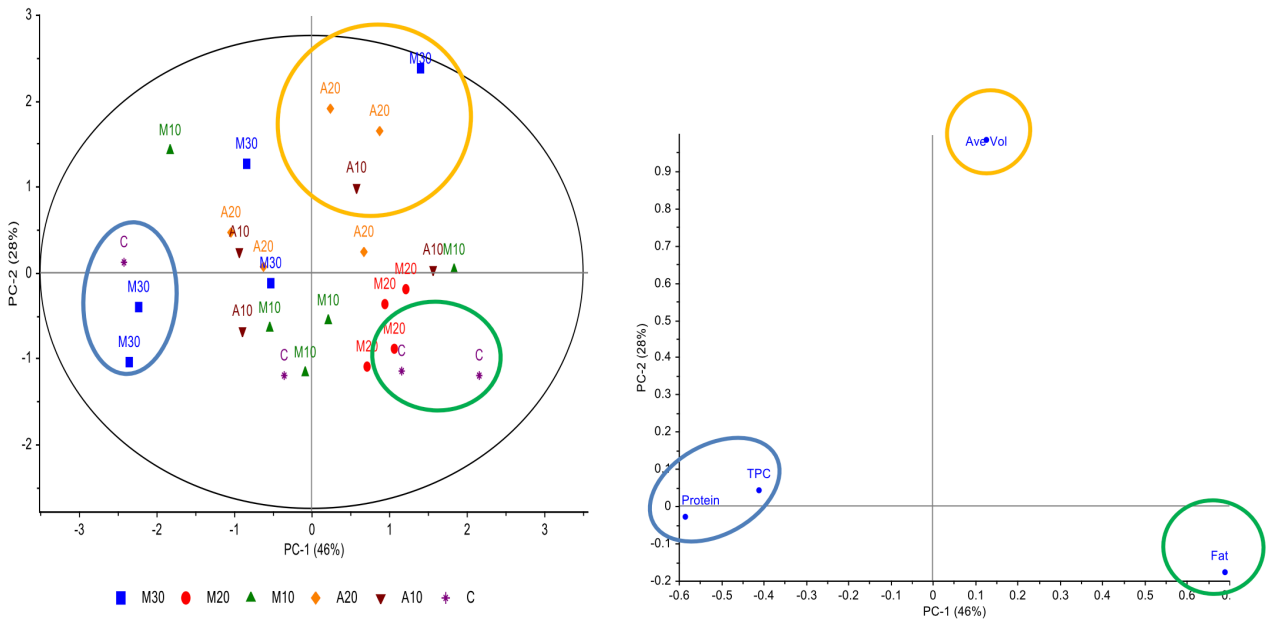


Fig. 8. PCA of milk samples on Day 30 (Month 1) and Day 60 (Month 2) based on the milk yield (mL), crude protein, fat and TPC contents. Scores plot (left):- C=Control (untreated); A=Ajwa DPP; M=Mariami DPP; Ave Vol=Mean milk yield Loadings plot (right):-Individual parameters

This comparison was made to envisage the possibility of having certain milk quality (crude protein, fat or TPC) in spite of the significant ($p < 0.05$) increased milk yield. The 'unsupervised' PCA protocol did not completely distinguish the milk samples into various groups. However, the analyses using 3D PCA illustration combining the control, A20 and M30 groups suggest that the samples can be separated into the two discrete clusters (Fig. 9). The A20 group correlated with the highest milk yielding data as seen in the Loadings plot, meanwhile the control group demonstrated having the highest content of fat. It can be assumed that MVA compared to bivariate analyses of parameters, in this case the milk yield and quality is a more comprehensive tool for understanding the effects of different supplementations on the lactating goats. Data visualisation using the 3D Scores plot explained 94% of the total variances among the milk quality data. Ametaj *et al*., [72] stated that the Loadings plot showed the variables responsible for the variation within the dataset, and the correlations among individual parameters corresponding to the first two eigenvalues (i.e., PC 1 and PC 2). Nevertheless, data from Table 5 was referred to for the PCA analysis as seen in Figs. 8 and 9, respectively. On the contrary, Sun *et al*., [75] defined milk quality focussing on its metabolic profiles in dairy cow's milk using PCA, and extended it to other biofluids including the rumen fluid, serum and urine. They applied the GC-TOF/MS and showed that the metabolic profiles of the four biofluids were significantly separated into clusters between the alfalfa hay (high quality) and corn stover (low quality) groups.

There were also other studies that deliberated on milk quality using PCA. Among them were studies on the association between the metabolite profile and technological properties of bovine milk from two dairy cow breeds [76], differences in milk from Holstein cows and other minor dairy animals [77], comparison between the mammary gland secretory tissue and milk serum in two goat breeds with dissimilar levels of tolerance to seasonal weight loss [21] and ovine milk with high somatic cell count [78].

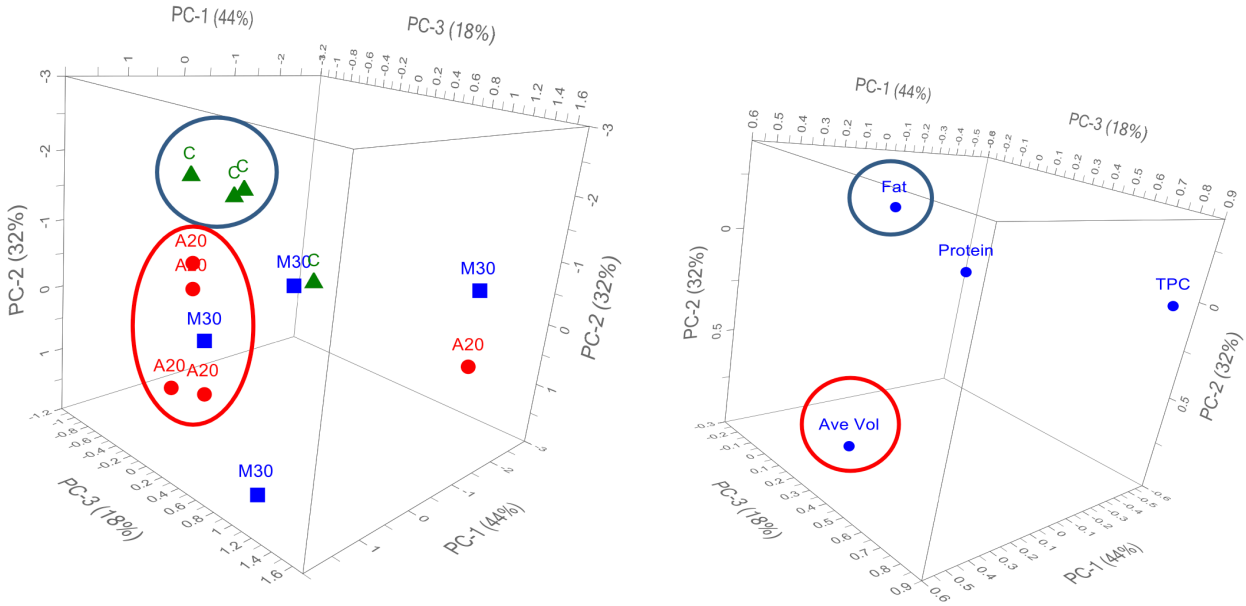


Fig. 9. 3D PCA visualization of different milk samples with the significant ($p < 0.05$) highest milk yield from A20 and M30 DPP groups compared to the Control focusing on several parameters (Milk yeld, crude protein, fat and TPC). Scores plot (left):-Individual samples; Loadings plot (right):-Individual parameters; C=control (untreated); A=Ajwa DPP; M=Mariami DPP

Table 5
Crude protein, fat and TPC contents (% Dry weight) of milk samples at day 30 (Month 1) and day 60 (Month 2) of the study period used for the 2D and 3D PCA

Milk quality parameters	Period	Treatments					
		Control	A10	A20	M10	M20	M30
Crude Protein	Month 1	21.42 ± 8.50	23.96 ± 6.14	27.13 ± 2.18	21.11 ± 4.00	19.83 ± 3.56	28.31 ± 1.24
	Month 2	23.65 ± 5.15	23.67 ± 6.05	22.09 ± 9.03	21.38 ± 9.07	23.25 ± 4.03	25.32 ± 4.55
Fat	Month 1	25.61 ± 14.42	16.53 ± 6.55	25.07 ± 4.90	23.95 ± 12.17	30.39 ± 5.22	20.88 ± 5.88
	Month 2	28.35 ± 6.29	22.50 ± 11.63	22.36 ± 3.81	23.72 ± 4.87	23.77 ± 6.77	26.23 ± 10.97
TPC	Month 1	0.32 ± 0.12	0.32 ± 0.11	0.34 ± 0.23	0.26 ± 0.05	0.28 ± 0.01	0.31 ± 0.06
	Month 2	0.24 ± 0.02	0.39 ± 0.20	0.23 ± 0.03	0.26 ± 0.05	0.28 ± 0.03	0.34 ± 0.13

Note: Data are mean ± SD of n=3 and is not significantly different ($p > 0.05$). TPC=Total phenolics contents. A=Ajwa DPP, M=Mariami DPP

3.2.2 Partial Least Squares (PLS)

In comparison to the PLS regression which was not generated by the Mariami DPP *vs*. control model, PLS of Ajwa DPP *vs*. control was successfully produced and depicted as in Fig. 10 but with a moderate correlation (R^2) value of 0.62 and weak validation (Q^2) value of 0.17. Prior to PLS, data were normalized to create the most homogenized data set as much as possible. It was indicated that the control was distinguished from the Ajwa DPP-treated goats postulating the predictive effects of the DPP supplementation. DPP doses had also some contribution to the relative clustering of milk samples with higher doses (20 g Ajwa DPP) being furthest from the control. Monakhova *et al*., [65] reported that by using PLS regression, their NMR spectra were correlated with nutrition information from labelling of lactose-free milk and milk substitutes based on either soy, oat or rice. Among the analysed variables (Milk yield, crude protein, fat and TPC), milk yield was made the dependent variable and the other parameters as the independent variables. Milk yield was significantly ($p < 0.05$) altered (Fig. 11) due to both milk crude protein and fat contribution to the PLS model as seen in Fig. 12.

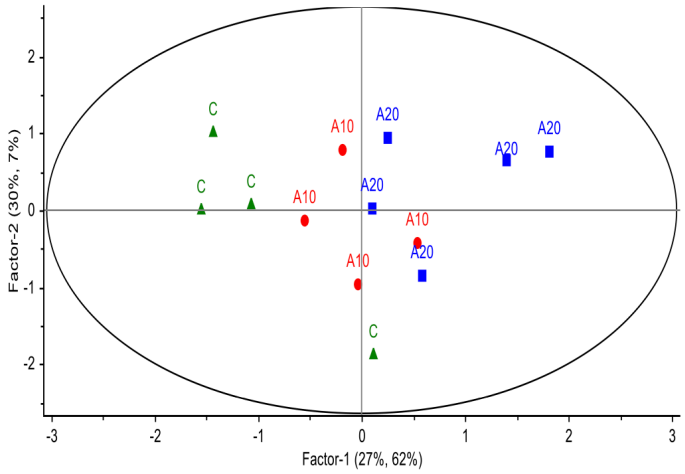


Fig. 10. PLS of Ajwa DPP groups *vs*. Control. C=control; A10=10 g of Ajwa DPP; A20=20 g of Ajwa DPP

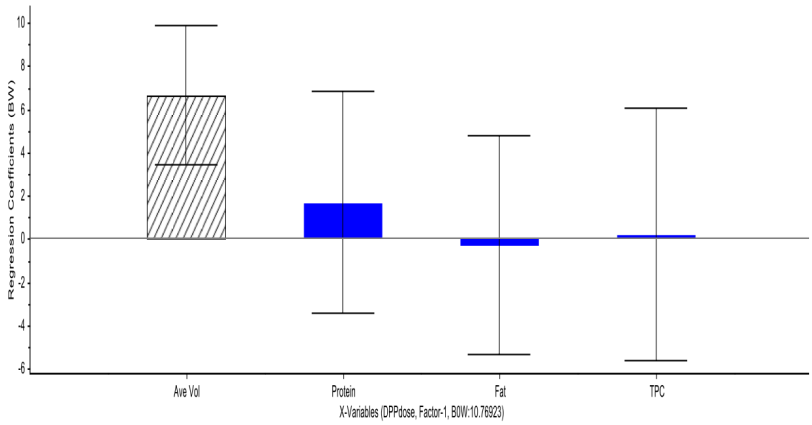


Fig. 11. Contributing factors in the PLS model; Lined bar=Significant ($p < 0.05$) affected by milk crude protein and fat

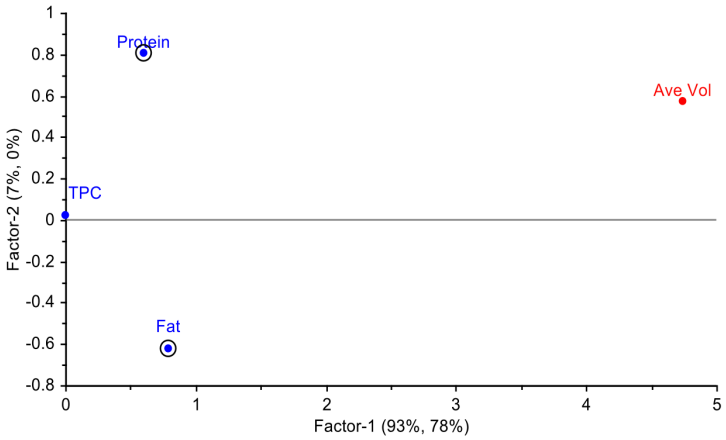


Fig. 12. PLS of mean milk yield prediction for Months 1, 2 and 3; R^2=0.78; Q^2=0.70; Ave Vol=Mean milk yield

4. Conclusion

It can be concluded that DPP supplementations to the lactating goats increased milk yield significantly ($p < 0.05$) for both A20 and M30 DPP treatments compared to the control but not in milk crude protein, fat, lactose and TPC contents in spite of the dry season indicating that dietary intake of DPP irrespective of dose, does not ($p > 0.05$) affect the milk quality. Furthermore, 2D and 3D PCA analyses using the multi-nutrient (Milk yield, crude protein, fat and TPC) parameters can be useful tools to investigate the effect of DPP supplementation on milk traits produced by the goats with A20 was assigned to the significant highest milk yield and control to the highest fat content in the 3D PCA presentation. PLS of Ajwa DPP *vs*. control was successfully generated but with a moderate correlation and weak validation (R^2=0.62; Q^2=0.17). Furthermore, the milk yield variable was significantly ($p < 0.05$) affected in the PLS model (R^2=0.78; Q^2=0.70), affected by both milk crude protein and fat parameters. Hence, DPP as an agricultural waste by-product and its supplementation at higher doses to the lactating goats can be a viable alternative to increase the milk productivity of small and local goat farmers for enhancing food security and sustainability.

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