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AGRO-INDUSTRIAL BY-PRODUCTS AS A FEEDING STRATEGY FOR PRODUCING FUNCTIONAL MILK

Ahmed A. Abd El-Maksoud[1]*, Mohamed A. Radwan[2], Hassan A. F. Rahmy[2], Fouad M. F. Elshaghabee[1], Ahmed M. Hamed[1]

[1] Dairy Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Animal Production Research Department, Faculty of Agriculture, Cairo University, Giza, Egypt

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ABSTRACT

Agro-industrial by-products contain valuable components such as polyphenols that can play a significant role in producing milk with specific properties through rumen fermentation. In Egypt, the buffalo milk chain has a potential market, which is larger than that for cow milk, especially in small villages. Therefore, this study aims to explore different feed ingredients from agro-waste for enhancing the functional properties of raw buffalo milk including polyphenols, flavonoids, vitamins A, C, α- tocopherol, and conjugated linoleic acid (CLA) contents. For this purpose, thirty dairy water buffalo (*Bubalus bubalis*) with a weight of about 520 kg were randomly divided into five groups of six animals each. The first group was fed a basal diet (silage) without agro-waste, while the other four groups were fed the basal diet after replacing 25% of the diet with different agro-wastes including barley (snack/bakery waste), sweet potato/carrot, biscuit/cake waste and tomato pomace. The feeding experimental period lasted 90 days, then milk samples (n = 150) were collected. Our results show that adding sweet potato/carrot or tomato pomace to the basal diet enhanced the contents of vitamins A, C, and phenolic compounds reflecting on the enhancement of the antioxidant capacity of raw buffalo milk. Concerning the CLA content, the milk samples collected from buffalo fed the basal diet fortified with tomato pomace and biscuit/cake waste had the highest CLA and α-tocopherol content. Therefore, this study recommends using the tested agro-waste ingredients for producing functional buffalo milk, especially for small-medium milk producers.

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ETHICS STATEMENT: All animal procedures were approved by Cairo University- Faculty of Agriculture and performed according to the Guide of animal care and use.

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АГРОПРОМЫШЛЕННЫЕ ОТХОДЫ КАК СТРАТЕГИЯ КОРМЛЕНИЯ ДЛЯ ПРОИЗВОДСТВА ФУНКЦИОНАЛЬНОГО МОЛОКА

Абд Эль-Максуд А. А.[1]*, Радван М. А.[2], Рахми Х. А.Ф.[2], Эльшагаби Ф. М.Ф.[1], Хамед А. М.[1]

[1] Кафедра науки о молоке, Сельскохозяйственный факультет, Каирский университет, Гиза, Египет
[2] Кафедра животноводства, Сельскохозяйственный факультет, Каирский университет, Гиза, Египет

КЛЮЧЕВЫЕ СЛОВА:
функциональное молоко, экологическая устойчивость, полифенолы, стратегии кормления, общие смешанные рационы

АННОТАЦИЯ

Агропромышленные отходы содержат ценные компоненты, такие как полифенолы, которые могут играть существенную роль в выработке молока со специфическими свойствами в результате рубцовой ферментации. В Египте цепь буйволиного молока имеет более крупный потенциальный рынок, чем коровьего молока, особенно в небольших деревнях. Таким образом, данное исследование нацелено на изучение различных кормовых ингредиентов от сельскохозяйственных отходов для повышения функциональных свойств сырого буйволиного молока, включая содержание полифенолов, флавоноидов, витаминов А, С, α-токоферола, и конъюгированной линолевой кислоты (CLA). С этой целью 30 молочных буйволов (*Bubalus bubalis*) весом приблизительно 520 кг были разделены на пять групп по шесть животных. Первая группа получала основной рацион (силос) без сельскохозяйственных отходов, в то время как остальные четыре группы получали основной рацион с заменой 25% рациона различными сельскохозяйственными отходами, включая ячмень (отходы снеков/хлебобулочных изделий), сладкий картофель/морковь, отходы печенья/пирожных и томатные выжимки. Экспериментальный период кормления длился 90 дней, а затем отбирали образцы молока (n = 150). Наши результаты показали, что добавление картофеля/моркови или томатных выжимок к основному рациону увеличивало содержание витаминов А, С и фенольных соединений, отражая усиление антиоксидантной способности сырого буйволиного молока. Что касается содержания CLA, то образцы молока, отобранные от буйволов, получавших основной рацион, фортифицированный томатными выжимками и отходами печенья/ пирожных имели наиболее высокое содержание CLA и α-токоферолов. Таким образом, данное исследование рекомендует использование тестированных ингредиентов сельскохозяйственных отходов для производства функционального буйволиного молока, особенно для мелких и средних производителей молока.

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1. Introduction

The increasing global population and the demand for nutritious food have led to a substantial increase in food waste (FW) generation. This waste from food manufacturing contributes to environmental pollution, sparking numerous efforts to investigate opportunities for transforming it into valuable resources [1]. One of the applications of food waste is its use as valuable and sustainable animal feed, especially, for livestock. This practice not only repurposes waste that would otherwise be considered valueless but also makes a significant contribution to environmental protection by reducing pollution and cutting production costs [1,2]. Therefore, breeders have turned to using food waste and agro-industrial by-products as alternatives to cereal-based concentrates, which tend to be less cost-intensive [3]. Moreover, the utilization of FW as animal feed offers a solution that not only addresses challenges related to FW management and food security but also reduces the demand for traditional feed development. On the other hand, these FW (natural by-products) with antimicrobial and antioxidant properties are recognized as potential sources of phenolic bioactive compounds, contributing to valuable health benefits [4]. Numerous studies have investigated the potential utilization of diverse manufacturing by-products. The increasing availability of different types of agro-industrial by-products has long raised interest in animal nutrition [5]. Agro-industrial wastes such as citrus peels are rich in polyphenols that can be used as feed ingredients [6] and they also can enhance the shelf-life of different types of dairy foods such as fermented milk [7].

Furthermore, microbial fermentation of agro-industrial wastes by rumen microbiota either in vitro or in vivo is a useful strategy for enhancing the production performance of ruminants [8]. Kaltenegger et al. [9] showed that bakery by-products used in the replacement of grains enhanced milk performance, modulated blood metabolic profile, and lowered the risk of rumen acidosis in dairy cows. The industrial tomato pomace (TP) as a by-product of the tomato industry contains 16% crude protein, 57% neutral detergent fiber, 44% acid detergent fiber, 8% ether extract (EE), and 24% lignin [10], and it showed a good acceptability by animals. Therefore, it has been used as a feed ingredient for small ruminants [11] and it can enhance lactation of goats [12]. Furthermore, addition of yeast as a feed ingredient in either active or heat-treated form can enhance rumen bacterial population and milk fermentation by starter cultures [13,14].

Recently, animal feed combining herbage and total mixed rations (TMR) is increasingly used in temperate regions for feeding ruminants, but little information is available regarding the effects on nutrient intake and digestion of this feeding management in beef cattle [15]. The objective of this study is to examine how incorporating various agricultural by-products (such as corn snack waste, sweet potato/carrot, biscuit/cake waste, or tomato pomace) into animal feed affects the improvement of the antioxidant activity, as well as the levels of vitamins (A and C), conjugated linoleic acid, and polyphenol contents in buffalo milk.

2. Material and Methods

2.1. Animals and feeding protocol

Animals were kept and handled at the farm of the Faculty of Agriculture, Cairo University, Giza Governorate during winter of 2023. Thirty dairy water buffalo (*Bubalus bubalis*) with a weight of about 520 kg were randomly divided into five groups of six animals each. The first group was fed a basal diet (silage) without agro-waste while the other four groups were fed the basal diet after replacing 25% of the diet with different agro-wastes including barley (snack /bakery waste), sweet potato / carrot, biscuit/cake waste and tomato pomace. The feeding experimental period lasted 90 days in the mid-lactation period. Animals were housed individually in soil-surfaced tie stalls (4.5 m^2/animal), under shade, without any bedding and with free access to water and diets.

2.2. Chemical characteristics of animal feed

The by-products and silage samples were analyzed in terms of moisture, crude protein, ash, and crude fiber. Dry matter (DM) was determined by drying samples at 105 °C for 3 hrs (Bio Gene Laboratory Air Oven, China), ash content was determined by ashing in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550 °C for 8 hrs. The nitrogen (N) content was estimated using the Kjeldahl method [16]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) determinations were based on the method of Van Soest et al. [16] using an ANKOM fiber analyzer. Feed was analyzed for the proximate composition according to [17] and nitrogen free extract was calculated by the equation: NFE = 100 — (% Moisture+% Ash+% Crude protein+% EE +% Crude fiber).

2.3. Chemical composition of milk samples

Milk samples were analyzed for moisture, lactose, fat, protein and ash as previously described by Olagunju et al. [18].

2.4. Determination of the total phenolic content

The Folin Ciocalteu reagent was used for the analysis of the total phenolic content [19] and the total flavonoid content was measured by a colorimetric assay according to Zhishen et al. [20].

2.5. Determination of vitamins A, C and alpha-tocopherol

Vitamins A and C were assessed in milk samples using a spectrophotometric method as described by [21,22]. For measuring vitamins A and C, the method was based on reduction of 2,6-dichloroindophenol by ascorbic acid (AA) in acidic solution whereas 2,6-dichloroindophenol had an absorption maximum at 518 nm and, when reduced by AA, this chromophore disappears. The UV/Vis spectrophotometer (Jenway, 6305) was adjusted at 620 nm for measuring vitamin A. In the case of alpha-tocopherol, milk lipids were extracted through a process involving centrifugation followed by alcohol-hexane extraction. The concentration of tocopherol was then calculated using the equation provided by Erickson and Dunkley [22].

2.6. Determination of conjugated linoleic acid (CLA)

CLA was determined according to Abd El-Salam et al. [23]. Briefly, fat was extracted with ethanol from different milk samples and the absorbance of the extract was measured at 233 nm. The CLA concentration was determined from a standard curve of CLA prepared by plotting the absorption at 233 nm of the standard pure CLA solutions (0.1–1 mg CLA mL^{-1}) in ethanol.

2.7. Identification and quantification of phenolic compounds fractions by high performance liquid chromatography (HPLC)

All milk samples were subjected to extraction by mixing with 10 ml of 80% methanol for 2 min, then centrifuged at 25.000xg for 10 min. The supernatant was decanted into a round-bottom flask. The pellet was re-suspended in 80% methanol (2x5ml) and centrifuged at 25.000xg for 10 min. The supernatants were combined, filtered through a 0.20 μm Millipore membrane filter and then brought up to a known volume (25 ml). Three milliliters were collected in a vial for subsequent HPLC analysis as previously described by Tafesh et al. [24]. Phenolic acid compounds were quantified using an Agilent-1260 series HPLC system (USA) and the phenolic acids were expressed as mg/kg sample on dry weight basis.

2.8. Analysis of fatty acid composition by gas chromatography (GC)

Firstly, fat from different milk samples was mixed with methanolic hydrogen chloride (15 mL HCl and 85 mL methanol) in a screw capped test tube and heated at 100 °C for 60 min until fat was fully dissolved. After that, the test tube was cooled down to room temperature. Then, 2 mL of distilled water and 2 mL of n-hexane were added to the tubes followed by vertexing (Vortex Mixer VX-200) at 1,500 rpm for 2 min. After vertexing, the tube was placed down for 5 min, or until two layers were separated. Then, the supernatant was poured into a tube containing 1 g of anhydrous sodium sulphate, which was transferred to GC vials and injected into the GC–MS (Agilent Technologies 5977A, USA) using a SP-2560 capillary column (75 m x 0.1 mm, DF 0.14 μm). GC–MS analysis was carried out according to Wang et al. [25].

2.9. Antioxidant activity

The antioxidant activity was measured by estimating the free radical scavenging activity using the 2,2`-diphenyl 1,1-picryl hydrazyl (DPPH) radical scavenging assay according to El-Maksoud et al. [26].

2.10. Ethics statement

These animals received humane care in compliance with the guidelines of the Institutional Animal Care and Use Committee of Cairo University (No CU II 6223; Nov 2023).

2.11. Statistical Analyses

Results were statistically analyzed with the SAS statistical package (version 9.4, SAS Institute, Cary, NC). Data were expressed as a mean of three replicates ± standard deviation (SD). Duncan test and one-way analysis of variance were used.

3. Results and discussion

3.1. Chemical composition and nutritional value of animal feed

In this study, data on production gathered from five farms revealed significant differences in feed consumption both among and within the five feed systems. The results of the analysis of the chemical composition (moisture, ash, crude fiber, crude protein, EE, and NFE) of different studied agro-industrial and food waste are presented in Table 1. It was found that high variability between the chemical composition of feed samples could explain the variability of the milk composition. Regarding the moisture content, the obtained results showed that the feed samples had the moisture content ranging from 6.14% to 12.34%. As regards the ash content, it was noticed that the snack/bakery waste samples had the highest value

(19.25%), while the silage sample had the lowest value (6.4%). Sweet potato/carrot samples had the highest value of crude fiber (26.42%), and the tomato pomace had the lowest value (7.19%). In relation to the fat content, the tomato pomace had the highest value (6.4%), while snack/bakery feed samples had the lowest value (1.87%). In regard to crude protein, the results showed that the silage feed samples had the highest values (12.01%) and the tomato pomace feed samples had the lowest concentration (7.64%). As for the NFE, the snack/bakery feed samples had the highest value (53.34%), while the sweet potato /carrot samples had the lowest value (39.1%). According to Soltan [27], the tomato by-product contained 91.72% dry matter, 20.13% crude protein, 7.92% EE, 29.33% crude fiber, 11.11% ash and 31.51% NFE. Alicata et al. [28] found that tomato waste contained 22.0% crude protein, 32.8% crude fiber and 19.5% EE. Khadr and Abdel-Fattah [29] demonstrated that the chemical composition of the tomato by-product was 94.6% dry matter, 20.93% crude protein, 10.22% EE, 37.74% crude fiber, and 14.37% ash. In [30], the proximate composition of tomato pulp was as follows: 89.8% DM, 19.9% crude protein, 16.1% EE, 13.6% NFE, and 4.88% ash. Soltan [27] found that the potato by-product meal (PBM) had 94.12% dry matter. Based on DM, PBM contained 8.02% crude protein, 3.6% EE, 18.13% crude fiber, 6.17% ash and 64.08% NFE. Karpukhin and Keita et al. [31] wrote that the chemical composition of the potato by-product contained about 25% dry matter, including 10 to 23% starch, 1.4–3.0% high-quality proteins. This indicates that our results are in accordance with previous studies as described above.

3.2. Chemical composition of milk samples

The feeding system markedly affected the chemical composition of milk. The moisture, lactose, fat, protein, and ash content of milk is presented in Table 2. Regarding the moisture content, it ranged between 85.1% in milk from the sweet potato/carrot group and 86.67% in milk from the tomato pomace group. The lactose content of the milk samples ranged from 4.13% for milk from the silage group to 5.42% for milk from the corn snack waste group. The milk samples from the biscuit/cake waste group had the highest fat content (8.03%), while the milk samples from the tomato pomace group had the lowest (6.49%). The protein content of the milk samples ranged from 3.18% to 3.90% for the tomato pomace group and biscuit/cake waste group, respectively. The ash content of the milk samples ranged from 0.70% to 0.92% for the sweet potato/carrot group and biscuit/cake waste group, respectively. Milk from the biscuit/cake waste feeding system was significantly higher in fat, protein, and ash, which makes biscuit/cake waste a potentially high-energy and nutrient-dense feed ingredient. On the other hand, milk of the tomato pomace system had the lowest levels of fat and protein.

However, it is important to note that the nutrient requirements of animals vary depending on species, age, and other factors. Thus, the best feed ingredient for one animal may not be the same as for another. Noted changes in the milk composition may be associated with both the lactation stage and the feeding system employed [32]. The intricacy of diverse farming systems is evident in the reports of differing and occasionally conflicting outcomes, attributed to the multitude of factors in production and feeding, along with their interactions that can impact both milk yield and composition [33].

3.3. Functional properties of milk samples

Functional foods have been defined as foods that provide an additional physiological benefit that may prevent disease or promote health and well-being. The total phenolic content, flavonoid content, CLA, vitamin C, vitamin A, and α-tocopherol in the milk samples are presented in Table 3. The total phenolic content (TPC) ranged between 2.37 and 8.93 mg/ml for the control and tomato pomace feeding system. Tomato pomaces are a great source of phenolic compounds, as well as non-phenolic compounds (benzyl alcohol, saturated and unsaturated fatty acids, carotenoids, and others), which have excellent redox properties [34]. The flavonoid content ranged between 5.92 and 28.18 µg/L in the milk samples from the control and biscuit/cake waste feed systems. The CLA content was in a range between 3.22 and 6.22 mg/g fat in milk from the snack/bakery waste group and tomato pomace group, respectively. Milk from the sweet potato / carrot group was significantly higher in vitamin C and vitamin A (1.08 and 181 µg/100g, respectively) compared to other milk samples. In recent times, the demand for and attention to orange-fleshed sweet potato are increasing due to the high concentrations of β-carotene and non-provitamin A carotenoids [35]. However, it was noticed that the content of α-tocopherol (1.38 mg/kg) in the milk samples from the biscuit/cake waste group was significantly higher than that in the other milk samples.

The experimental values (means and SD for n = 150) with different superscript letters in the same column indicate significant difference ($P \leqslant 0.05$).

3.4. Phenolic fractions of milk samples

In the HPLC analysis of phenolic acids in the milk samples (Figure 1), it is crucial to underscore the significance of cattle feed in shaping the

Table 1. **Mean chemical composition of the TMR samples based on agro-waste collected from five farms in Giza and Beheira governorate**

Таблица 1. **Средний химический состав образцов общих смешанных рационов (TMR) на основе сельскохозяйственных отходов, собранных на пяти фермах в провинциях Гиза и Бухейра**

Feed samples TMR	Moisture %	Ash %	Crude fiber	Ether extract	Crude protein	NFE
Control	10.93 ± 0.35^b	6.40 ± 0.17^d	20.15 ± 0.24^b	4.18 ± 0.02^b	12.01 ± 0.41^a	46.33 ± 0.29^d
Snack/bakery waste	8.15 ± 0.16^c	19.25 ± 0.30^a	9.49 ± 0.27^d	1.87 ± 0.01^c	7.90 ± 0.10^d	53.34 ± 0.18^b
SP/C	11.17 ± 0.24^b	12.00 ± 0.11^b	26.42 ± 0.46^a	2.74 ± 0.01^b	8.57 ± 0.26^c	39.10 ± 0.44^e
Biscuit/cake waste	6.14 ± 1.28^d	9.28 ± 0.06^c	17.95 ± 0.41^c	6.23 ± 0.05^a	9.53 ± 0.14^b	50.87 ± 1.25^c
Tomato pomace	12.34 ± 0.16^a	6.96 ± 0.34^d	7.19 ± 0.50^e	6.47 ± 0.11^a	7.64 ± 0.66^d	59.4 ± 1.03^a

TMR: Total mixed ration; NFE: nitrogen-free extract; SP/C: sweet potato/carrot.
The experimental values (means and SD for n = 150) with small letters are significantly different ($P \leqslant 0.05$).

Table 2. **Mean chemical composition of the milk samples (n = 150) collected from five feed systems in Giza and Beheira governorate**

Таблица 2. **Средний химический состав образцов молока (n = 150), полученных от пяти систем кормления в провинциях Гиза и Бухейра**

Feed samples	Moisture	Carb	Fat	Protein	Ash
Control	85.66 ± 0.45^b	4.13 ± 0.24^c	6.53 ± 0.02^d	3.32 ± 0.21^c	0.72 ± 0.06^c
Snack/bakery waste	85.28 ± 0.50^b	5.42 ± 0.36^a	6.87 ± 0.21^c	3.20 ± 0.10^c	0.78 ± 0.04^{bc}
SP/C	85.17 ± 0.16^b	4.55 ± 0.27^{bc}	7.74 ± 0.13^b	3.57 ± 0.26^b	0.70 ± 0.05^c
Biscuit/cake waste	85.91 ± 0.81^{ab}	4.91 ± 0.41^{ab}	8.03 ± 0.15^a	3.90 ± 0.11^a	0.92 ± 0.03^a
Tomato pomace	86.67 ± 0.24^a	5.25 ± 0.23^a	6.49 ± 0.16^d	3.18 ± 0.15^c	0.81 ± 0.04^b

SP/C: sweet potato/carrot.
The experimental values (means and SD for n = 150) with different superscript letters in the same column indicate significant difference ($P \leqslant 0.05$).

Table 3. **Mean chemical composition of milk samples (n = 150) collected from five farms in Giza and Beheira governorates**

Таблица 3. **Средний химический состав образцов молока (n = 150), полученных на пяти фермах в провинциях Гиза и Бухейра**

Feed samples	TPC Rutin Equivalent mg/ml	Flavonoids µg/L	CLA mg/g fat	Vitamin C µg/100 g	Vitamin A mg/100 g	α-Tocopherol mg/kg
Control*	2.37 ± 0.01^d	5.92 ± 0.08^e	3.49 ± 0.16^d	0.52 ± 0.03^c	142.5 ± 3.25^c	0.22 ± 0.00^d
Snack/bakery waste	4.15 ± 0.16^c	9.23±0.06c	3.22 ± 0.04^d	0.55 ± 0.07^c	155.1 ± 2.05^b	0.14 ± 0.01^e
SP/C	8.17 ± 0.24^a	12.05 ± 0.11^b	4.64 ± 0.15^c	1.08 ± 0.08^a	181.3 ± 2.61^a	0.81 ± 0.02^c
Biscuit/cake waste	6.04 ± 1.28^b	28.18 ± 0.32^a	5.43 ± 0.24^b	0.62 ± 0.06^b	136.2 ± 1.10^d	1.38 ± 0.01^a
Tomato pomace	8.93 ± 0.35^a	8.64 ± 0.05^d	6.22 ± 0.09^a	0.79 ± 0.10^b	157.9 ± 2.42^b	0.92 ± 0.06^b

* Feed sample collected from the basal diet; SP/C: sweet potato/carrot.

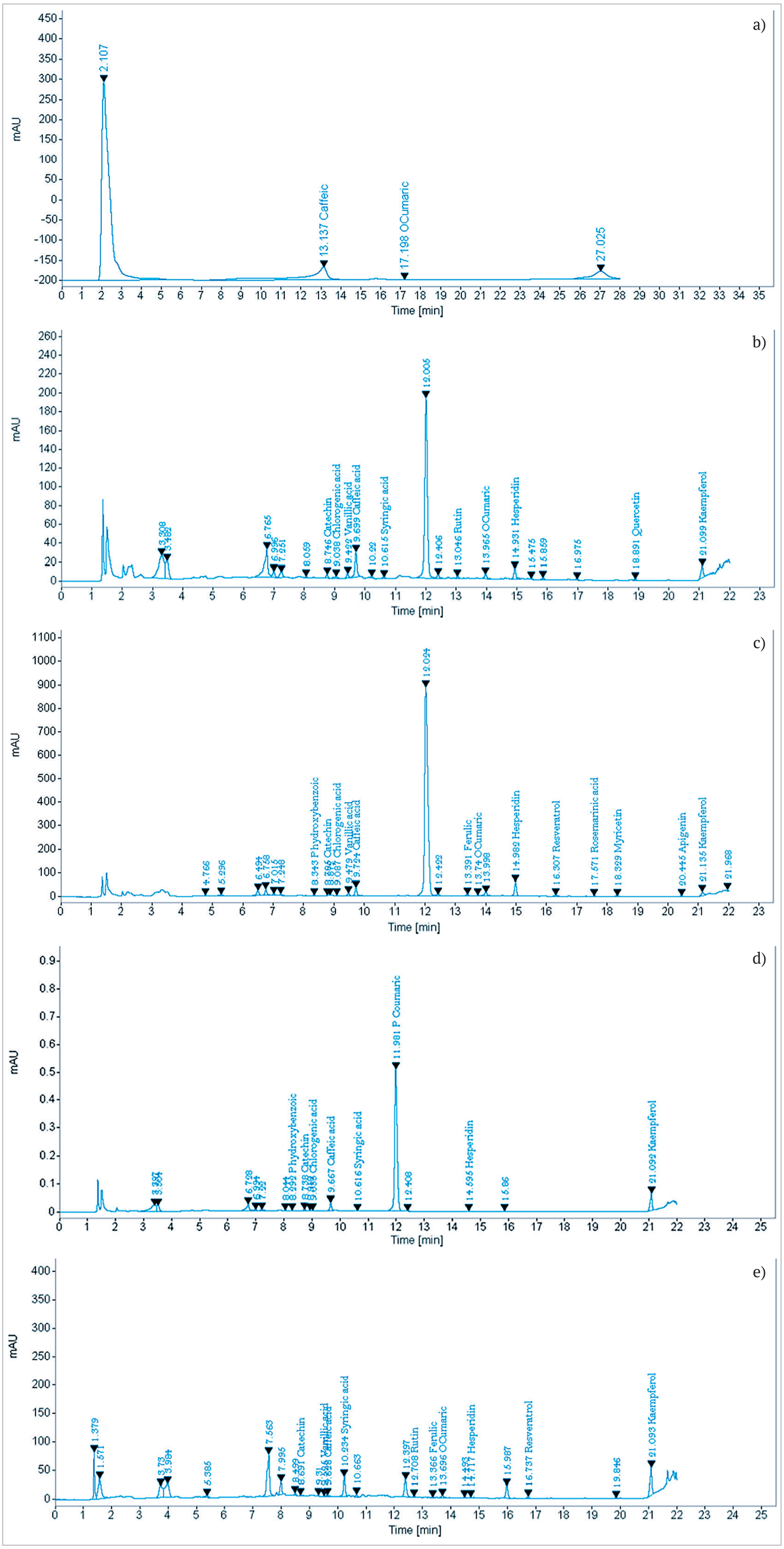

Figure 1. **HPLC chromatogram of phenolic fractions for different milk samples: Control (a) SP/C (b); Snack/bakery waste (c); Biscuit/cake waste (d); Tomato pomace (e). SP/C: sweet potato/carrot**

Рисунок 1. **ВЭЖХ хроматограмма фенольных фракций для различных образцов молока: контроль (a) сладкий картофель/морковь (b); отходы снеков/хлебобулочных изделий (c); отходы печенья/пирожных (d); томатные выжимки (e)** composition of milk. Table 4 presents the phenolic fractions identified in the milk samples collected from various feed groups, highlighting the impact of different feeding regimes on the phenolic profile. Each waste source demonstrated a unique composition of compounds, showcasing variability in their quantities and types when compared with the control. Milk collected from the control group (normal feed) exhibited the absence of all phenolic acids except for caffeic and o-coumaric acids. Conversely, various phenolic fractions were identified in milk from all groups fed with food industrial waste. Some compounds were present in the specific milk samples based on the waste sources. Notably, the biscuit /cake waste feeding group demonstrated the highest content of catechin and kaempferol (5.44 and 19.95 mg/kg), while p-coumaric acid (119.70 mg/kg) was detected only in milk from this group. Similarly, rutin was present only in the sweet potato/carrot and tomato pomace groups, while hesperidin was dominant in the snack/bakery waste group. Additionally, there were moderate concentrations of vanillic acid (3.74 mg/kg) and resveratrol (4.288 mg/kg). P-Hydroxybenzoic acid (1.33 mg/kg) and catechin (1.64 mg/kg) were also observed, albeit in relatively lower amounts. Regarding the milk samples derived from the tomato pomace group, a notable feature was the high content of syringic acid (7.79 mg/kg), which stands out with a substantial quantity. Additionally, there was a presence of a smaller amount of resveratrol (2.51 mg/kg). These results align with the total phenolic content observed in the milk samples (Table 3), which was influenced by the incorporation of waste products in animal feeding.

Table. 4. **Phenolic fractions (mg/ kg) of milk samples of different feed systems**

Таблица 4. **Фенольные фракции (мг/кг) образцов молока различных систем кормления**

Phenolic fractions	Control	Snack/ bakery waste	Sweet potato/ carrot	Biscuit/ cake waste	Tomato pomace
P-Hydroxybenzoic acid	—	1.334	—	1.199	—
Catechin	—	1.640	3.475	5.439	1.93
Chlorogenic acid	—	1.183	3.048	1.755	—
Vanillic acid	—	3.741	1.604	—	0.656
Caffeic acid	7.10	8.617	7.499	7.16	0.745
Syringic acid	—	—	0.459	0.314	7.79
P-Coumaric acid	—	—	—	119.698	—
Rutin	—	—	0.681	—	0.598
Ferulic acid	—	5.056	—	—	0.89
O-Coumaric acid	0.08	0.458	0.808		0.637
Hesperidin	—	24.588	5.079	0.726	1.116
Quercitin	—	—	2.919	-	—
Resveratrol	—	4.288	—	—	2.504
Rosemarinic acid	—	2.181	—	—	—
Myrcitin	—	0.718	—	—	—
Apigenin	—	0.119	—	—	—
Kaempferol	—	6.38	4.233	19.95	15.71

3.5. Fatty acid content of milk samples

The ability to modify milk fat is largely dependent on the efficiency with which fatty acids are transferred from a diet to milk. Table 5 shows the composition of fatty acids in different milk samples collected from different feed programs based on agro-waste additives. Each row represents a specific type of fatty acid, while each column corresponds to different milk samples (control, snack/bakery waste, sweet potato/carrot, biscuit/cake waste, and tomato pomace). For instance, observe how butyric acid (C4) or palmitic acid (C16) differed among the samples. The butyric acid content ranged from 2.06 to 2.86 across different milk samples. Despite minor variations, the butyric acid levels remained relatively consistent among the samples, suggesting a stable presence regardless of the different feed program. However, notable differences existed in the caproic acid levels. Explore how these variations in feed might relate to the specific additives used in animal feeding. The caprylic acid content ranged from 0.36 to 0.55 across different milk samples. Some fatty acids showed relatively consistent levels across the samples (e. g., butyric, caprylic and capric acids), while others exhibited more significant variations (e. g., myristic, palmitic and stearic acids).

On the other hand, both oleic and linoleic acids showed considerable variability among the samples, indicating potential influences from the additives. These variations in unsaturated fatty acids (USFA) (oleic and linoleic acids) highlight potential changes in the nutritional content and quality across different milk samples based on the type of agro-waste. As shown in Table 5, using the agro-waste in the feed for animals improved the quality and nutritional value of secreted milk, especially in the case of tomato pomace (USFA content: 27.19 and 5.34% of oleic and linoleic acids, respectively).

Table. 5. **Fatty acid profile (mol%) of milk fat of different milk samples**

Таблица 5. **Жирнокислотный профиль (моль%) молочного жира различных образцов молока**

Fatty acids	Control	Snack/ bakery waste	Sweet potato/ carrot	Biscuit/ cake waste	Tomato pomace
Butyric (C4)	2.33	2.06	2.14	2.86	2.71
Caproic (C6)	1.48	1.44	1.06	1.30	0.86
Caprylic (C8)	0.45	0.41	0.36	0.55	0.39
Capric (C10)	0.91	1.09	1.20	0.69	0.94
Lauric (C12)	1.88	1.81	2.13	1.94	1.75
Myristic (C14)	9.13	11.08	13.35	12.61	9.04
Palmitic (C16)	32.38	38.97	30.42	30.52	29.67
Stearic (C18)	16.01	17.08	13.53	20.34	15.72
Oleic (C18:1; cis-9)	21.5	19.62	27.34	24.05	27.19
Linoleic (C18:2; cis-9, trans-12)	2.17	3.81	1.55	3.29	5.34

3.6. Antioxidant properties of milk samples

Data in Figure 2 show that fortification of silage with sweet potato/carrot and tomato pomace at 25% resulted in a significant enhancement in levels of the antioxidant activity as measured by DPPH and ABTS free radical scavenging. Our results are in accordance with the results obtained by [36,37] whereas feeding cattle or Egyptian buffalo with sweet potato/ carrot and tomato pomace enhanced health and production aspects. Therefore, both ingredients can be used as a natural source of antioxidants for improving the antioxidant capacity of buffalo milk through increasing the phenolic content (Table 4). For instance, it is well-established that caffeic acid and kaempferol possess antioxidant properties, potentially reducing oxidative stress and offering neuroprotective effects [38,39]. Moreover, studies by Rudrapal et al. [40] indicate that p-coumaric acid exhibits potential anticancer effects.

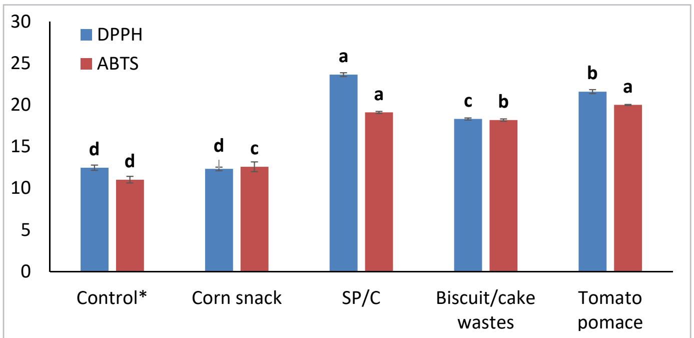

Figure 2. **DPPH and ABTS radical scavenging assay of the milk samples obtained from different feed systems. SP/C: Sweet potato/Carrot. Measurements with different letters are significantly different ($p < 0.05$).**

Рисунок 2. **Анализ образцов молока, полученных в различных системах кормления, используя метод захвата радикалов DPPH и ABTS SP/C: сладкий картофель/морковь. Результаты измерений с разными буквами достоверно отличаются ($p < 0,05$).**

4. Conclusion

Egyptian buffalo milk needs improvement, especially when we know that raw buffalo milk is more preferable than cow milk for consumers. In order to develop this chain, our study focuses on using agro-waste ingredients for feeding strategies of water buffalo. We conclude that sweet potato/carrot or tomato pomace can be used as an ingredient for feeding buffalo along the dairy chain for enhancing the functional properties of raw buffalo milk, producing milk rich in phenolic compounds and with the antioxidant activity that serves different sustainable development goals (SDG 3 and 12). It is believed that dietary polyphenols/flavonoids exert the powerful antioxidant action for protection against reactive oxygen species (ROS)/cellular oxidative stress (OS) towards the prevention of OS-related pathological conditions or diseases.

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AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Ahmed A. Abd El-Maksoud, Associate Professor, Dairy Science Department, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt Tel.: +2–0111–701–16–41 E-mail: ahmed_ali@cu.edu.eg ORCID: https://orcid.org/0000-0001-6631-5900 * corresponding author	**Абд Эль-Максуд А. А.,** доцент, Кафедра науки о молоке, Сельскохозяйственный факультет, Каирский Университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0111–701–16–41 E-mail: ahmed_ali@cu.edu.eg ORCID: https://orcid.org/0000-0001-6631-5900 * автор для контактов
Mohamed A. Radwan, Associate Professor, Animal Production Department, Cairo University 1 Gamaa Street, 12613, Giza, Egypt Tel.: +2–0155–792–22–88 E-mail: m.radwan883@agr.cu.edu.eg ORCID: https://orcid.org/0000-0001-8793-9659	**Мохамед А. Радван,** доцент, Кафедра животноводства, Каирский университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0155–792–22–88 E-mail: m.radwan883@agr.cu.edu.eg ORCID: https://orcid.org/0000-0001-8793-9659
Hassan A. F. Rahmy, Lecturer, Animal Production Department, Cairo University 1 Gamaa Street, 12613, Giza, Egypt Tel.: +2–0100–046–01–86 E-mail: Hasan.awny@agr.cu.edu.eg ORCID: https://orcid.org/0009-0004-5409-5823	**Хасан А. Ф. Рахми,** преподаватель, Кафедра животноводства, Каирскоий университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0100–046–01–86 E-mail: Hasan.awny@agr.cu.edu.eg ORCID: https://orcid.org/0009-0004-5409-5823
Fouad M. F. Elshaghabee, Associate Professor, Dairy Science Department, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt Tel.: +2–0103–035–41–37 E-mail: elshaghabee@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-9576-3945	**Фуад М. Ф. Эльшагаби,** доцент, Кафедра науки о молоке, Сельскохозяйственный факультет, Каирский Университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0103–035–41–37 E-mail: elshaghabee@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-9576-3945
Ahmed M. Hamed, Associate Professor, Dairy Science Department, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt Tel.: +2–0115–376–32–41 E-mail: ahmed.hamed@agr.cu.edu.eg ORCID: https://orcid.org/0000-0003-3055-7486	**Ахмед М. Хамед,** доцент, Кафедра науки о молоке, Сельскохозяйственный факультет, Каирский Университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0115–376–32–41 E-mail: ahmed.hamed@agr.cu.edu.eg ORCID: https://orcid.org/0000-0003-3055-7486

Contribution	**Критерии авторства**
Authors equally relevant to the writing of the manuscript, and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.