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RESEARCH ARTICLE
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Chemical composition, anti-oxidative activity and *in vitro* dry matter degradability of Kinnow mandarin fruit waste

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Abstract

Aim: Fruit processing and consumption yield a significant amount of by-products as waste, which can be used as potential nutrient suppliers for livestock. "Kinnow" (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora) is one of the most important citrus fruit crops of North Indian States. Its residues are rich in carbohydrates but poor in protein and account for approximately 55-60% of the raw weight of the fruit. Present study assessed the chemical composition and anti-oxidative activity of Kinnow mandarin fruit waste (KMW) and scrutinized the impact of dietary incorporation of variable levels of KMW on *in vitro* dry matter digestibility (IVDMD).

Materials and Methods: Sun dried and ground KMW was analyzed for proximate composition, fibre fractions and calcium and phosphorus content. Antioxidant potential of KMW as total phenolic count and 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was assayed in an alcoholic extract of KMW. The effect of inclusion of KMW at variable levels (0-40%) in the isonitrogenous concentrate mixtures on *in vitro* degradability of composite feed (concentrate mixture:Wheat straw; 40:60) was also carried out.

Results: KMW after sun-drying contained 92.05% dry matter. The crude protein content of 7.60% indicates it being marginal in protein content, whereas nitrogen free extract content of 73.69% suggests that it is primarily a carbonaceous feedstuff. This observation was also supported by low neutral detergent fiber and acid detergent fiber content of 26.35% and 19.50%, respectively. High calcium content (0.92%) vis-à-vis low phosphorus content (0.08%), resulted in wide Ca:P ratio (11.5) in KMW. High anti-oxidative potential of KMW is indicated by total phenolic content values of 17.1±1.04 mg gallic acid equivalents/g and DPPH free radicle scavenging activity 96.2 µg/ml (effective concentration 50). Mean IVDMD% of all the composite rations was found to be comparable ($p>0.05$) irrespective of the level of KMW inclusion, indicating no negative effect of KMW inclusion on *in vitro* nutrient utilization.

Conclusion: KMW is a carbonaceous feedstuff and may be incorporated in ruminant ration up to the level of 40% (w/w basis) without affecting *in vitro* degradability.

Keywords: antioxidant, *in vitro* dry matter digestibility, Kinnow mandarin waste, unconventional feedstuff.

Introduction

Fruit production and consumption has seen tremendous growth in last few years. The area under fruit cultivation in India is 6.98 million hectares with a total production of 81.28 million tonnes [1]. Processing and consumption of fruits yield a significant amount of by-products as waste. It has been demonstrated that these fruit by-products can be potential nutrient suppliers especially energy for livestock [2]. These are available free of cost, are rich in micro-nutrients and can positively affect livestock health due to their high anti-oxidative activity. Livestock feeding regimen based on these by-products also offers two important advantages; it helps in diminishing dependence of livestock on grains and eliminates the need for costly waste management programs [3].

Citrus fruits are principally consumed by humans as fresh fruit or processed juice. After juice is extracted from the fruit, there remains a residue comprised of peel (flavedo and albedo), pulp (juice sac residue), rag (membranes and cores) and seeds. These components, either individually or in various combinations, are available for utilization as ruminant feedstuffs [4].

"Kinnow," a hybrid between King and willow mandarins (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora) is one of the most important citrus fruit crops in North Indian States [5]. Jammu and Kashmir produces 20,810 MT of citrus fruit [1], majority of which is produced in Jammu division and out of which a considerable share is that of Kinnow mandarins. Kinnow

residues are rich in carbohydrates but poor in protein and account for approximately 55-60% of the weight of the raw fruit [6].

Present study was conducted to assess the chemical composition and anti-oxidative activity of Kinnow mandarin fruit waste (KMW) and to scrutinize the impact of dietary incorporation of variable levels of KMW on *in vitro* dry matter digestibility (IVDMD).

Materials and Methods

Study material

KMW was collected from local juice vendors of Jammu city and nearby areas in the evening. Immediately after collection in plastic buckets, KMW was brought to the laboratory and sun dried by spreading out on polythene sheets and was frequently turned around to ensure quick and uniform drying. After about 16 h of sun-drying, waste was pulverized through a laboratory grinder to form a coarse powder.

Procedures

Proximate analysis and calcium and phosphorus content estimation of pulverized KMW sample was done as per [7], whereas fiber fractions (neutral detergent fiber [NDF] and acid detergent fiber [ADF]) were analyzed as per [8].

Antioxidant potential of KMW was assayed in an alcoholic extract of KMW. Extract was prepared by refluxing 10 g of dried and ground KMW with 100 ml of 70% ethanol at 72°C for 121 min. The extract was filtered through Whatman No.4 filter paper and was re-dissolved in the appropriate amount of 70% ethanol to get the final concentration of 5.0 mg/ml.

Total phenolic content (TPC) in the prepared KMW extract was determined using the Folin–Ciocalteu reagent [9]. Result was expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE/g). The ability of KMW extract to scavenge the 1-diphenyl-2-picrylhydrazyl (DPPH) (2,2-diphenyl-1-picryl-hydrazylhydrate) free radical was measured [10]. DPPH solution (40 mg/L) was prepared by dissolving DPPH in 100% methanol. KMW extract was then added to 3 ml of the DPPH solution, and the absorbance was determined at 515 nm after 10, 30, 60, and 120 min.

The effect of inclusion of KMW at variable levels (0-40%) in the isonitrogenous concentrate mixtures on *in vitro* degradability of composite feed (concentrate mixture:Wheat straw; 40:60) was carried out [11]. Ingredient composition of different iso-nitrogenous concentrate mixtures formulated for *in vitro* study along with the percent *in vitro* IVDMD is detailed in Table-1.

Statistical analysis

Statistical analysis of the data generated was performed using a personal computer. Generalized linear model analysis of variance for data generated by *in vitro* trial and one-way analysis of variance for chemical composition data was conducted [12].

Results

The proximate composition and fiber fractions of KMW used in this study are shown in Table-2.

KMW after sun-drying contained 92.05% dry matter (DM). On DM basis, KMW contained 7.60% crude protein (CP) and 73.69% nitrogen free extract (NFE) along with NDF and ADF content of 26.35% and 19.50%, respectively.

The TPC was found to be 17.1±1.04 mg GAE/g of KMW, whereas the DPPH free radicle scavenging activity of KMW was determined as median effective concentration 50 and was found to be 96.2 µg/ml.

In vitro DM digestibility of composite diet containing variable levels of KMW in iso-nitrogenous concentrate mixtures are shown in Table-1. The mean IVDMD% of all the composite rations was found to be comparable ($p>0.05$) irrespective of the level of KMW inclusion. The percent IVDMD of ration containing 40% KMW (w/w) in concentrate mixture

Table-1: *In vitro* dry matter digestibility of composite ration comprising of variable levels of KMW.

Iso-nitrogenous rations containing variable levels of KMW[1]	IVDMD (%)
Ration 1 (KMW 0%, Maize 29.5%, WB 29.5%, MOC 38%, MM 2%, Salt 1%)	52.99±2.66
Ration 2 (KMW 10%, Maize 24.0%, WB 24.0%, MOC 39%, MM 2%, Salt 1%)	52.74±2.66
Ration 3 (KMW 15%, Maize 21.0%, WB 21.0%, MOC 40%, MM 2%, Salt 1%)	52.96±1.70
Ration 4 (KMW 20%, Maize 18.0%, WB 18.0%, MOC 41%, MM 2%, Salt 1%)	52.66±1.99
Ration 5 (KMW 25%, Maize 15.5%, WB 15.5%, MOC 41%, MM 2%, Salt 1%)	51.44±2.14
Ration 6 (KMW 30%, Maize 13.0%, WB 13.0%, MOC 41%, MM 2%, Salt 1%)	54.24±2.69
Ration 7 (KMW-35%, Maize 10.0%, WB 10.0%, MOC 42%, MM 2%, Salt 1%)	53.96±2.72
Ration 8 (KMW-40%, Maize 7.0%, WB 7.0%, MOC 43%, MM 2%, Salt 1%)	50.37±3.52

[1]All rations were incubated in rumen liquor along with wheat straw in 40:60 proportion. KMW=Kinnow mandarin waste; WB=Wheat bran, MOC=Mustard oil cake, MM=Mineral mixture

Table-2: Chemical composition of KMW.

Attribute	Percentage*
Moisture	92.05±3.71
OM	95.77±3.94
CP	7.60±0.07
EE	3.57±0.01
CF	10.92±0.46
Total ash	4.23±0.37
AIA	0.50±0.01
NFE	73.69±1.34
NDF	26.35±2.68
ADF	19.50±2.01
Calcium	0.92±0.01
Phosphorus	0.08±0.001

*All values except moisture are expressed on DM basis. DM=Dry matter, OM=Organic matter, CP=Crude protein, EE=Ether extract, CF=Crude fibre, AIA=Acid insoluble ash, NFE=Nitrogen free extract, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, KMW=Kinnow mandarin waste

was 50.37±3.52, which was similar (p>0.05) to that (52.99±2.66) of the ration having conventional concentrate (0% KMW).

Discussion

High moisture content of citrus waste has always restricted their use as feedstuff [4]. Moisture content of <10% achieved after sun-drying for 16 h in this study indicate that this method and duration is sufficient to dry KMW for further processing and storage, although frequent turning is essential to achieve this level of drying.

The CP content of 7.60% indicate it being marginal in protein content, whereas NFE content of 73.69% suggests that it is primarily a carbonaceous feedstuff and based on the composition, it can be considered as a replacement candidate for starchy feedstuffs used in conventional ration. This observation was also supported by low NDF and ADF content. High calcium content (0.92%) vis-à-vis low phosphorus content (0.08%), resulted in wide Ca:P ratio (11.5) in KMW. This finding is similar to the observations from other citrus fruit wastes [4]. This wide calcium: Phosphorus ratio needs to be corrected by using phosphorus supplements while formulating the practical rations.

The chemical composition analytical data relevant to animal nutrition and pertaining to KMW is scanty in the referred literature. The proximate composition and fiber fractions values of KMW obtained in this study are in agreement with the values available for waste of other citrus fruits [13,14]. A comparative analysis with the cellulose (8.8%), hemicellulose (4.4%), lignin (3.7%), protein (12.2%) and ash (5.9%) content of dried Kinnow pulp [15] indicate that Kinnow pulp is having less ADF and NDF content and more crude protein and ash content as compared to Kinnow peel.

TPC values found in the present study are similar to the values previously reported for Kinnow peels [16]. Phenolics or polyphenols have received considerable attention for their physiological function, including antioxidant, anti-mutagenic and anti-tumour activities. These are believed to account for a major portion of the antioxidant activity in many plants. A linear correlation between phenolic content and antioxidant ability in fruits and vegetables has been demonstrated [17], however in contrast, it has also been reported that Kinnow peel extract with lower TPC in comparison to extracts from litchi and grape residues exhibited higher free scavenging ability [16]. The TPC content of KMW was found to be higher than that reported for edible portions of other common fruits [18]. Similar observation was reported by Khalid *et al*. [19].

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants [20]. The DPPH free radicle scavenging activity of KMW estimated in the present study indicate higher antioxidant activity than other common fruit residues like grape and litchi residues [16] and pomegranate peel [21].

The comparable mean IVDMD% of all the composite rations irrespective of the level of KMW indicate that KMW inclusion does not hamper the nutrient degradability and its utilization is similar to that of other carbonaceous feedstuffs (maize and wheat bran [WB]). Similar observations were reported [22,23] in studies on *in vitro* fermentation of another citrus pulp. It has been suggested that supplementation of forages with citrus fruit waste that are rich in pectin or highly degradable NDF usually has a less negative effect on the rumen environment, and thus on cellulolytic activity, than supplementation with starch-or sugar-rich feeds [4,24]. When citrus fruit waste substitutes for starchy feeds, DM digestibility remains unaffected [25,26]. This is in agreement with the findings of the present study, where the isonitrogenous concentrate mixtures tested for IVDMD were prepared mainly by replacement of maize and WB (starchy feedstuffs) from the conventional concentrate by KMW.

It is quite possible that even higher levels of KMW inclusion in concentrate mixture would not have impacted IVDMD values of composite ration; however, formulating a balanced diet in terms of calorie protein ratio would have become increasingly difficult with increasing levels of KMW.

Conclusion

KMW seems to be an ideal energy supplement for ruminants besides providing beneficial effects over the health as evident by high anti-oxidative potential. As per the results of present study, 40% inclusion level of KMW in concentrate mixture seems to be appropriate under *in vitro* trial; however, *in vivo* feeding trials are required to validate the *in vitro* results.

Authors' Contributions

AR and RKS designed the research program. RK conducted the research work. AK and PR helped in conducting laboratory analysis. All authors contributed in the preparation of the manuscript. AR revised the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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