EVALUATION OF THE NUTRITIONAL POTENTIAL OF SAFFLOWER (CARTHAMUS TINCTORIUS L.) LEAVES. SEED AND CAKE AFTER OIL EXTRACTION TO BE USED AS ANIMAL FEED

MASTER OF SCIENCE IN CROP SCIENCE

(HORTICULTURE)

BY

DINEO PHUDUHUDU

SEPTEMBER 2017

UNIVERSITY OF BOTSWANA

BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

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EVALUATION OF THE NUTRITIONAL POTENTIAL OF SAFFLOWER (*Carthamus tinctorius* L.) LEAVES, SEED AND CAKE AFTER OIL EXTRACTION TO BE USED AS ANIMAL FEED

A research Dissertation submitted to the University of Botswana in partial fulfillment of the requirements for the Degree of Master of Science in Crop Science (Horticulture)

By

Dineo Phuduhudu (ID: 200700124)

September 2017

Main-Supervisor: Prof. V. E. Emongor

Co-Supervisor: Dr. O. Oagile

Botswana University of Agriculture and Natural resources

Department of Crop Science

CERTIFICATION

PROF. VALENTINO EMONGOR 25/09/2017

Main- Supervisor Name and Signature ..... Date

DR. OTSOSENG OAGILE 25-09-2017

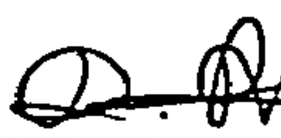
Co- Supervisor Name and Signature ..... Date

UTLWANG BATLANG 25/9/2017

Head of Department Name and Signature ..... Date

STATEMENT OF ORIGINALITY

I, the undersigned hereby declare that the work contained in this thesis was carried out by the author at the Botswana University of Agriculture and Natural Resources, between January 2015 and June 2017. This work contains no material previously published by another person or material which has been accepted by any other degree or certificate of any university except where due acknowledgement and reference has been made in the text.



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ACKNOWLEDGEMENTS

I would like to thank the almighty God for the strength, health and patience he gave me throughout this project. I wish to also express my sincere gratitude to my supervisor Professor Vallantino Emongor for the support, valuable time, guidance and advices he gave me from the proposal development, research work to thesis writing, as well as the Animal Science and Crop Science technicians for demonstrating some experimental procedures, which enabled me to carry out laboratory experiments. Foremost, I would like to extend my heartfelt gratitude to the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding this research.

DEDICATION

I dedicate this dissertation manuscript to my late father, Lieutenant Kebalaole Wire Phuduhudu, who believed in me that I can go to greater heights academically if I put it to heart, may his soul rest in peace; my mother, Mrs Margaret Phuduhudu who devoted her time to take care of my son while I was busy carrying out my research; my fiancé Mr Onalenna Kereilwe who devoted his time to assist me with throughout thesis writing.

ABSTRACT

Safflower is an annual oil seed crop adapted to arid and saline regions of the world, is a potentially novel food and medicine source, which has not been fully explored in the last century. Safflower's ability to forage for sub-soil moisture with its vigorous tap root, tolerance to salinity, adaptability to wide range of temperatures, improved oil content and its versatility to produce oil high in linoleic and oleic fatty acids makes it a viable alternative to current crops grown in the more marginal cropping areas around the world. This study was carried out to evaluate the nutritional potential of the safflower leaves, seed and cake after oil extraction to be used as animal feed. Nine safflower genotypes were evaluated in a completely randomized block design with three replications in the Botswana University of Agriculture and Natural Resources, Notwane Farm under sandy loam soils. The results of the study showed that safflower genotypes significantly ($P < 0.05$) differed in oil, crude protein (CP), dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ash and mineral contents of the leaves, whole seeds and cake after oil extraction. The cake CP, NDF, ADF, ADL and ash contents significantly ($P < 0.05$) varied between 19.3–22.5, 54.6–61.2, 45.0–50.7, 18.0–20.8, and 1.10–1.60%, respectively, depending on genotype and growing season. While the seed mineral content significantly ($P < 0.05$) varied between 6.98–7.90 mg/g P, 10.68–12.91 mg/g K, 8.78–10.61 mg/g Ca, 4.45–4.99 mg/g Mg, 90–120 ppm Zn, 70–90 µg/g Fe, 40–50 µg/g Mn, and 90–130 µg/g Cu, respectively, depending on genotype and growing season. Significant ($P < 0.05$) differences were also observed in the seed oil content, DM, CP, NDF, ADF, ADL and ash which varied between 26.13–42.17, 91.9–96.1, 16.3–19.1, 42.6–50.3, 39.7–48, 13.5–20.7, and 0.95–1.41%, respectively, depending on genotype and growing season. The seed mineral content significantly ($P < 0.05$) differed between 5.47–7.87 mg/g P, 8.44–12.26 mg/g K, 11.33–9.45 mg/g Ca, 4.37–5.55 mg/g Mg, 3.24–3.57 mg/g Na, 90–120 µg/g Zn, 50–80 µg/g Fe, 30–50

µg/g Mn, and 130–170 µg/g Cu, respectively, depending on genotype and growing season. The leaf DM, CP, ND, ADF, ADL and ash significantly ($P < 0.05$) varied between 88.1–91.2, 21.1–27.7, 20.5–26.2, 26.5–32.7, 6.7–10.7, and 0.89–1.13%, respectively, depending on genotype and growing season. The leaf mineral content significantly ($P < 0.05$) differed between 3.31–4.95 mg/g P, 56.13–66.54 mg/g K, 10.61–16.51 mg/g Ca, 3.91–4.92 mg/g Mg, 0.51–0.69 mg/g Na, 70–90 µg/g Zn, 310–460 µg/g Fe, 280–380 µg/g Mn, and 6.3–8.3 µg/g Cu, respectively, depending on genotype and growing season. In conclusion, safflower has a great potential to be grown in Botswana as an oilseed crop and source of animal feed.

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LIST OF ABBREVIATIONS

AAAD- Apparent amino acid digestibility

ADF- Acid detergent fiber

ADL- Acid detergent lignin

AME- Average metabolized energy

ANOVA- Analysis of variance

AOAC- Association of Official Agricultural Chemist

BUAN- Botswana University of Agriculture and Natural Resources

Ca- Calcium

CF- Crude fiber

CLA- Conjugated linoleic acid

CP- Crude protein

CSC- Clean safflower cake

Cu- Copper

EDTA- Ethylene dimethyl tetra acid

ESC-Extruded safflower cake

Fe- Iron

FSH- Follicle stimulating hormone

GE- Gross energy

HSC- Hull extruded safflower cake

ICP-MS- Inductively coupled plasma mass spectrometry

IGF-1- Insulin growth factor 1

IVMOD- In vivo organic dry matter

K- Potassium

LH- Luteinizing hormone

LSD- Least significant difference

Mg- Magnesium

Mn- Manganese

MUFA- Mono-unsaturated fatty acid

N- Nitrogen

Na- Sodium

NDF- Neutral detergent fiber

NRC- National research council

OM- Organic matter

P- Phosphorus

RCBD- Randomised complete block design

SAS- Statistical analysis system

SBM 44- Soy bean meal 44

SESC- Solvent extracted safflower cake

SFA- Saturated fatty acid

TAAD- True amino acid digestibility

TMR- Total mixed ration

UFA- Unsaturated fatty acid

WLC- Whole linted-cotton seed

WSS- Whole safflower seed

Zn- Zinc

CHAPTER 1

1.0 INTRODUCTION

1.1 Background information

Safflower (*Carthamus tinctorius* L.) is a herbaceous, thistle-like annual plant belonging to the family Compositae or Asteraceae. It has a chromosome number of 2n = 24 (Knowles 1989; Lopez, 1989; Ekin, 2005). Safflower can grow 30 to 210 cm tall with a strong central stem. The plant is highly branched and terminates into a flower head; each branch usually has from 1 to 5 flower heads containing 15 to 20 seeds per head surrounded by a cluster of leafy spiny bracts (Emongor, 2010). The leaves are spiny, waxy and oblong. Flowers are yellow, orange or red flowers (Emongor, 2010). The seeds (achenes) are white, smooth, shining and 6–7 mm long (Helm *et al.*, 2012). The seeds are also four sided with a thick pericarp, weighing about 0.031 to 0.0589g (Emongor, 2010). Arslan (2007) reported a seed weight of 0.3767–0.4444 g. Safflower plant also has a strong tap root penetrating to 2–3 m soils with adequate depth. The deep root system in safflower helps the plant to extract the water and nutrients from much deeper layers of soil, compared to other crop plants, and thus makes it an ideal plant for rain-fed cropping systems (Singh and Nimbkar, 1993).

Safflower is one of humanity's oldest crops, with its use reported over 2,200 years ago. Safflower seeds were reported in Egyptian tombs over 4,000 years ago. However, safflower cultivation remained a backyard crop for personal use and as a result it remained a minor and neglected crop with world seed production in 1989 estimated at 908,000 tons (Rowland, 1993; Gyulai, 1996). Interest in cultivation of safflower has increased because of increased demand for vegetable oil for biodiesel and edible oil (Mailer *et al.*, 2008). Interest in cultivating safflower as

source of edible oil has further been stimulated since the identification of safflower oil as a rich source of polyunsaturated essential fatty acid linoleic acid (70–87%) and monounsaturated fatty acid oleic acid (11–80%) (Murthy and Anjani, 2008; Aghamohammadreza *et al.*, 2013). Linoleic acid has been shown to offer nutritional and therapeutic benefits such as prevention of coronary heart disease, arteriosclerosis, high blood pressure and hyper lipaemia (Wang and Li, 1985; Cosge *et al.*, 2007). Safflower is an oilseed crop which is mainly grown in semi-arid regions; it is used as cooking oil, colouring and flavouring foods, vegetable, industrial oil, spice, bird feed, cut-flower, fodder and forage crop, making herbal tea, making dyes for the textile industry, and medicinal purposes (Ekin, 2005; Emongor, 2010).

1.2 Origin and distribution

De- Candolle (2007) proposed that safflower originated in Southern Asia and is known to have been cultivated in China, India, Persia and Egypt from prehistoric times. It is native to the Middle East (Vavilove, 1951; Knowles, 1969; Ashri, 1975; Weiss, 2000; Ekin, 2005) and it's tolerant to saline conditions and to drought stresses (Francois and Berstein, 1964; Bassiri *et al.*, 1977; Weiss, 2000; Bassil and Kaffka, 2002). During middle ages it was cultivated in Italy, France and Spain and soon after discovery of America, the Spanish took it to Mexico and then to Venezuela and Colombia. It was introduced into United States in 1925 from the Mediterranean region and is now grown in over 600 countries all over the world.

1.3 Agronomical characteristics

It is ideal to grow safflower in April or early May in Botswana so that the time of flowering and seed development is not synchronized with the time of extreme low temperature (<7 °C) in winter (Emongor *et al.*, 2013). Safflower is very susceptible to frost injury during winter (Bergman and Kandel, 2013). Seed germination takes 3-8 days depending on temperature and

soil moisture; and germination occurs at temperature as low as 2–5°C (Emongor, 2010). Seed germination is followed by a slow growing rosette stage, during which numerous leaves are produced near ground level and a tap root system which penetrate deep into the soil (Dajue and Mündel, 1996; Carapetian, 2001; Emongor, 2010). Rosette stage may last form 20 to 39 days from emergence depending on the variety, climatic conditions (temperature and photoperiod) and growing conditions (Dajue and Mündel, 1996; Weiss, 2000; Emongor, 2010; Emongor *et al.*, 2013). The rosette stage is considered as one of the main phenological phases in the growth of safflower plant (Tanaka *et al.*, 1997; Uslu, 1997; Carapetian, 2001). Generally, in most varieties of safflower, the rosette stage is short, but it may be prolonged by environmental factors such as low temperatures or short days. Lower temperature during winter lengthens the rosette period, which is associated with higher grain yields (Insua, 1986; Salera, 1997; Emongor *et al.*, 2013). Most cultivars with a short rosette stage can resist temperatures as low as -6.6 °C, some germplasm with long rosette stage have been found and tested in China that can withstand temperatures as low as -15 °C during the early stages of growth. The cold requirement of long rosette safflower causes these plants to develop a very dense clump of leaves in spring planting with total lack of stem development or delayed stem elongation (Li, 1989; Li *et al.*, 1997; Emongor *et al.*, 2013). Carapetian (2001) compared 11 selections of late rosette (winter type) safflower germplasm with 12 selections of spring type safflower and he found that late rosette safflower developed a very hardy and frost resistant seedling in fall planting at about the six-leaf stage with extensive root growth that could stand temperatures of -20 °C. Immediately after the rosette stage, stem elongation begins forming sturdy branches about 45 to 75 cm long. At the end of each stem is a flower head enclosed by miniature of leaves called bracts. The bloom stage lasts for 14 to 21 days, depending on the variety, plant density and availability of moisture.

Flowering begins from the central stem and spreads outward (Emongor, 2010). It takes two weeks to dry the crop for harvesting after seed maturity and the seeds would have matured within 30 to 35 days of flowering (Dajue and Mündel, 1996; Mündel *et al.*, 2004). It is harvested when most of the leaves have turned brown and only a hint of green remains on the bracts of the latest flowering head (Mündel *et al.*, 2004).

Safflower grows best in temperate areas, where wheat and barley do well. It is frost tolerant but the tolerance differs greatly with varieties (Dajue and Mündel, 1996; Mündel *et al.*, 2004). In general, frost affects flowering and seed development stages, thus reducing yields and quality (Li *et al.*, 1997; Emongor, 2010). It is a long-day plant requiring a photoperiod of about 14 hours. It is shade and weed intolerant (Blackshaw *et al.*, 1990). It will not grow as a weed because other wild plants overshadow it before it becomes established. The plant grows best in deep fertile, sandy or clay loam soils with good drainage and water holding capacity (Weiss, 2000). Soil moisture is needed from planting through flowering stages. It may be grown under irrigation or as a dry land crop. Often grown following a wetland crop, such as rice, on high water table land without additional irrigation. For irrigation, 914-1067 mm of water is needed and under dry land conditions 300-635 mm are required (Weiss, 2000; Bassil and Kaffka, 2002). It also requires annual temperature between 6.3 to 27.5 °C and soil pH of 5.4-8.2 (Raghu and Sharma, 1978). However, safflower can grow in a wide range of temperatures from -7 to 40°C provided during elongation and flowering stages of growth and development (Emongor, 2010). Moreover, when producing safflower care should be taken as it is susceptible to many diseases caused by fungi, bacteria, viruses and other disorders due to the environmental stress. The diseases of safflower include; *Sclerotinia* head rot, *Alternaria* blight, rust and damping off of seedlings (Dajue and Mündel, 1996). Its pests of include cutworms, wireworms, seed corn maggots, painted lady

butterfly (*Vanessa cardui*), grasshoppers (*Melanoplus bivittatus*), lygus bugs, leafhoppers (*Empoasca* spp.), and thrips (Dajue and Mündel, 1996; Mundel *et al.*, 2004).

1.4 Utilisation of safflower

Safflower is a multipurpose oilseed crop grown mainly for its seed, which is used as edible oil (high quality), birdseed, animal feed and industrial oil (Weiss, 2000; Dordas and Sioulas, 2008; Dordas and Sioulas, 2009; Istanbulluoglu, 2009; Emongor, 2010; Khalili *et al.*, 2014). It is also used as cut flowers, vegetables, spice, for colouring, flavouring foods and making margarine, as medicines, for making red and yellow dyes for the textile industry, cosmetics (shampoos, hair and face cream, body lotion and perfumes), herbal teas and high quality paint (Shouchun, *et al.*, 1993; Bergland *et al.*, 2007; Emongor, 2010). The uses of safflower have been recorded in China approximately 2,200 years ago (Dajue and Mündel, 1996). Traditionally, safflower was grown for its seeds, for colouring and flavouring foods, as medicines and for making red and yellow dyes, especially before cheaper aniline dyes became available (Weiss, 1971). In Egypt, dye from safflower was used to colour cotton and silk as well as ceremonial ointment used in religious ceremonies and to anoint mummies prior to binding. Safflower seeds and packets and garlands of florets have been found with 4000-year-old mummies (Weiss, 1971).

1.4.1 Oil production

Food producers and industries use safflower oil. The seed oil content ranges between 20 to 45% depending on the variety and growing environment (Dajue and Mündel, 1996). The seed oil contains 1.5% myristic (with lauric and lower acids), 3% palmitic, 1% stearic, 0.5% arachidic (with trace of lignoceric), 11-80% oleic, and 70-88% linoleic acids depending on genotype and growing conditions (Keys, 1976; Dajue and Mündel, 1996; Murthy and Anjani, 2008; Aghamohammadreza *et al.*, 2013). Linoleic acid has been shown to offer nutritional and

therapeutic benefits such as prevention of coronary heart disease, arteriosclerosis, high blood pressure, hyper lipaemia and low density lipoprotein (Wang and Li, 1985; Smith, 1996; Cosge *et al.*, 2007). Safflower oil is considered a healthier option than using sunflower oil. They are two types of safflower that produce different types of oil, that which is high in monounsaturated fatty acids (oleic acid) and that which is high in polyunsaturated fatty acids (linoleic acid) (Heuze *et al.*, 2012). The two types of oil are considered high-quality edible oil, and public awareness about this health topic has made safflower an important crop for vegetable oil production (Huntrods, 2012).

1.4.2 Vegetable

Safflower leaves are eaten as vegetables (Weiss, 1983) and they are rich in carotene, riboflavin, vitamins A and C, iron, phosphorus and calcium (Singh and Nimbkar, 2006). Though the leaves are low in calories (33 cal), they have a good water content and rich fiber. Other nutrient content of leaves include: 2 g protein, 1g fat, 1g minerals, 4g carbohydrates, 185 mg Ca, 35 mg P and 6 mg of Fe per 100 g of fresh leaves (Keys, 1976). The slight bitterness of leaves help to control blood sugar level, thus beneficial for diabetic people (Gopalan *et al.*, 2004). Precisely, the bitter taste dilates arteries hence increasing the blood flow and oxygenation of tissues resulting in reduced hypertension (Deng, 1988).

1.4.3 Medicinal uses

Clinically, safflower is reported to dilate arteries, reduce hypertension and increases blood flow and hence, oxygenation of tissues (Deng, 1988; Wang and Yili; 1985). It is also reported to inhibit thrombus formation and over time, dissolve thrombi. Many prescriptions for invigorating blood circulation, especially those for treatment of heart disease, include safflower along with other herbs and have been used in the treatment of many diseases (Wang and Yili, 1985).

Cardiovascular disease treatment is the main use of safflower because it invigorates blood circulation. In 83% of patients with coronary disease, blood cholesterol levels were reduced after six weeks of treatment (Wang and Yili, 1985). Experiments with dogs showed that injections of safflower reduced damage done to the heart muscle by an infarction. Heart arrhythmia and hypertension were reduced by safflower treatment three times a day for four weeks (Wang *et al.*, 1978; Wang and Yili; 1985). Treatment of cerebral thrombosis with safflower was reported to improve and lower blood pressure in over 90% of patients (Wang and Yili, 1985; Yu, 1987). Safflower decoctions have been used successfully for the treatment of male sterility (Qin, 1990) and dead sperm excess disease (Qin, 1990). Treatment with safflower resulted in pregnancy in 56 of 77 infertile women who had been infertile for 1.5-10 years (Zhou, 1986).

1.4.4 Dying purposes

High linoleic acid safflower oil has an important use in the paint industry. Before 1960's in the USA, the oil was used mainly as a base for superior quality paints. It is used as a drying agent in paints and varnishes because of its non-yellowing characteristic (Bergland *et al.*, 2007). Safflower oil is also used in making paint in place of linseed oil. In textiles, dried flowers are used as natural dyes. Natural dyes from plants are getting more important nowadays because of their natural and fashion trends. The colourful matter in safflower is carthamin which is benzoquinone-based (Darroch, 1990). It has a dye of flavonoid type. Hydrophilic fibres like cotton, wool and others can be dyed with safflower dye because it is a direct dye. Safflower yellow or red pigments are safe for cosmetics colourings such as hair cream, shampoo, face cream, perfume or body lotions (Shouchun *et al.*, 1993).

1.5 Justification

Botswana's location in the sub-tropical high pressure belt of southern hemisphere, in the interior of southern Africa and away from oceanic influence makes it experience low rainfall and high temperatures in summer. There is high inter-annual variability of rainfall and drought is a recurring element of Botswana's climate (Emongor, 2009). Drought adversely affects the already fragile food and agricultural situation in the country and seriously impairs the rural economy and socio-cultural structures. Due to the erratic, unreliable and poorly distributed rainfall accompanied by high temperatures, water becomes the most limiting factor to agricultural production in Botswana (Emongor, 2009). In Botswana, the annual precipitation and evapotranspiration ranges between 200–650 mm and 1800–3000 mm, respectively, depending on season (Emongor *et al.*, 2008). Therefore, growing a drought and winter tolerant crop such as safflower will improve food security, reduce reliance on feed imports and improve income levels of farmers in Botswana.

In many countries safflower has been cultivated for its oil potential, but research has shown that the safflower cake have high protein content which could be beneficial in feeding or supplementing domesticated animals (BarT-al *et al.*, 2008). Many people are not considering the oil meal because of its unpalatability, but research has shown that palatability can be improved by mixing it with other feed concentrates, thus be used to improve the quality of meat of livestock as well as performance (Smith, 1996). Furthermore, safflower is a multi-purpose crop which can be used to diversify the economy of Botswana as it can also be grown as a cut flower, vegetable, birdseed and medicinal purposes in addition to oil and livestock feeds.

1.6 Objectives

The overall goal of this study was to evaluate the nutritional potential of safflower leaves, whole seed and cake after oil extraction to be used as animal feed with the aim of mitigating the effects of drought and climate change, improve food security (as it can be eaten as a vegetable), increase income and social welfare of farmers in Botswana, and to reduce reliance on animal feed importation.

The specific objectives of the study were to evaluate:

1) The nutritional potential of safflower leaves, whole seed and cake after oil extraction, to be used as animal feed.
2) To determine the effect of genotype and seasonal change on the nutrition of safflower leaves, whole seed and cake, after oil extraction.

1.6.1 Hypotheses

H_{01} = Safflower leaves, whole seed and cake after oil extraction has no nutritional potential.

H_{02} = Safflower genotypes and season have no influence on the nutritional composition of leaves, whole seed and cake after oil extraction.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Safflower as animal feed

2.1.1 Leaves

Safflower leaves can be grazed by animals and stored as hay or silage (Bar-Tal *et al.*, 2008). Its leaves are palatable with feed value and yields similar or better than oats and alfalfa (Smith, 1996; Wichman, 1996). It makes an acceptable livestock forage if cut at or just after bloom (Berglund *et al.*, 2007). Grazed safflower has been shown to support satisfactory growth rates in Australian steers (French *et al.*, 1988) and improved fertility in Canadian ewes (Stanford *et al.*, 2001). Apart from the cake, safflower plant can be cut at vegetative stage and made into good quality silage for livestock feed, although seed yield and quality of the seeds would be greatly reduced (Weinberg *et al.*, 2002). Livestock prefers safflower made into silage prior to spine development because they are more susceptible to mouth ulceration due to spines and lack the ability to select the most nutritious feed particles. Mouth irritations were not observed in sheep and goats, thus they could be fed to safflower cut after the bloom as hay. Full bloom safflower was found to be superior to alfalfa grass hay for mature ewes (Stanford *et al.*, 2001).

According to research by Dixon *et al.* (2003), feeding young sheep with rations based on low quality grass hay or cereal straw, supplementation by safflower cake permitted higher live weight gain and increased wool growth more than barley or urea supplement. In hay-fed lambs with 18 kg body weight, adding 350 g/day of concentrate mix with 25 % safflower cake resulted in an improvement of intake, digestibility, feed efficiency and body gain weight (Dessie *et al.*, 2010). The improvement was due to the high concentration of metabolize energy and crude protein

found in safflower cake, which alleviates under-nutrition of hay diets or cereal straws (Dixon *et al.*, 2003). After harvesting the field crops in arid areas, safflower can be planted as pasture for ruminant animals to lengthen their period of grazing. Safflower pastures are adequate for growing ruminants which have moderate requirements for pasture quality (Landau *et al.*, 2005). Grazed safflower has been shown to sustain satisfactory growth rates in Australian steers (French *et al.*, 1988). A safflower monoculture at the pre-blooming stage can be used safely as the only feed for grazing sheep (Landau *et al.*, 2004), and the intake of safflower green fodder, cut to a 30 cm height, has exceeded maintenance requirements for energy and protein in sheep (Vonghia *et al.*, 1992). Spineless safflower cultivars, which could be used as fodder with dry-matter (DM) yields of up to 22 ton/ha, and a high DM digestibility for safflower hay fed to heifers, have been introduced (Leshem *et al.*, 2001). It has also been reported to improve fertility in Canadian ewes, in comparison to alfalfa-grass hay (Stanford *et al.*, 2001). Safflower hay, given ad-libitum, has successfully been used as the only food for late-pregnant dairy cows (Landau *et al.*, 2004).

The *in vivo* organic dry matter (IVOMD) of safflower is closely related to the stage of maturity of the plant. As the plant ages, their nutritive value decreases, thus decreasing the IVOMD (Peiretti, 2009). Safflower utilized as fodder crop has shown a similar daily DM intake and digestibility values to those of a vetch–oat mixture when it was offered to rams and its gross energy (GE) content was around 18.0 MJ/kg DM (Vonghia *et al.*, 1992). Landau *et al.* (2004) measured the digestibility of a safflower hay product for pregnant dry cows when it was fed as the only feed. The *in vivo* digestibility of the DM from the safflower hay was 723 g/kg, while the *in vitro* DM digestibilities (IVDMD) of the ingested hay, leaves, stems, and orts were 646, 729, 546, and 505 g/kg, respectively (Peiretti, 2009).

2.1.2 Cake

2.1.2.1 Types of cake

Safflower cake is made from the residue that remains after oil extraction. Safflower cake can either be dehulled (decorticated) or hulled (undecorticated). Dehulled safflower cake refers to the cake with reduced or removed hulls before extraction of oil while hulled cake has complete hulls. The quality of the safflower cake is variable and depends on the amount of hulls and the extent of the oil extraction (Dajue and Mündel, 1996; Dschaak *et al.*, 2007; Jacob, 2015). Safflower oil can be obtained from the seeds by cold-pressing, expeller pressing or solvent extraction. Solvent extraction is more effective at oil removal than mechanical extraction (Jacob, 2015). Dehulling improves crushing efficiency during oil extraction, but the removal of hulls is expensive. Hulled (undecorticated) safflower cake has the protein and fiber contents of 20-25% and up to 60%, respectively (Voicu *et al.*, 2009; Jacob, 2015). In cakes produced from dehulled (decorticated) safflower seeds, the protein and fiber contents is about 35-50% and 10-15% fiber, respectively (Gohl, 1982; Voicu *et al.*, 2009; Jacob, 2015). The hull is the main source of fiber in the seed, so the level of crude fiber in safflower cake varies depending on the level of hulls remaining. Fiber can be 30% to 40% in undecorticated cakes and as low as 10% in decorticated cakes (Dajue and Mündel, 1996). The safflower cake from dehulled seeds can be used in compound feeds for pigs and poultry as a source for supplemental amino acids lysine, methionine and cysteine (Voicu *et al.*, 2009). The safflower cake with high fiber content can be used for ruminant feed (Voicu *et al.*, 2009).

2.1.2.2 Nutritional value

A study carried out by Farran *et al.* (2010) showed that the chemical composition of safflower cake depends more in the method of extraction. The results showed that partial dehulling of safflower seeds reduces fiber content in oil-extracted cakes from 35.3% hull extruded safflower cake (HSC) to 13% in partially extruded safflower cake (ESC) and solvent extracted safflower cake (SESC). Extensive removal of the kernel hulls before oil extraction further reduced fiber to 2.44% as in extruded clean safflower cake (CSC) making its protein source more suitable to be incorporated in broiler diets. Hexane extraction was more effective than cold extrusion in eliminating kernel oil because ether extract level was the lowest (1.59%) in SESC. A similar reduction in ether extract was reported by Zablan *et al.* (1963) when acetone extraction was used to prepare partially decorticated safflower cake in comparison with mechanical expelling. Hexane extraction reduced oil content from 8% in ESC to 1.59% in SESC without changing crude fiber level but increasing crude protein (CP) and nitrogen-free extract. Partial or extensive dehulling as in ESC, SESC, and CSC doubled percentage of ash but highly reduced their nitrogen-free extract in comparison with HSC (Farran *et al.*, 2010). The proximate analysis results of SESC, except for its higher CP, are comparable with those of SESC reported in NRC (1994). As a result of extensive dehulling and cold extrusion, the level of CP was increased from 20.5% in HSC to 55% in CSC (Farran *et al.*, 2010).

Research conducted to assess the nutritional composition of safflower cake, whole seed cake, showed that safflower cake obtained by expeller extraction contained 93.2% dry matter (DM) as fed, 24.8% crude protein (CP), 33.3% crude fiber (CF), 42.3% acid detergent fiber (ADF), 44.85% neutral detergent fiber (NDF), 9.4% ether extract, 4.4% ash and 21.4 MJ/kg energy (Heuze *et al.*, 2012). The mineral content were 2.7 g/kg calcium (Ca), 6.7 g/kg, phosphorus (P),

2.8 g/kg magnesium (Mg), 64 mg/kg zinc (Zn), 24 mg/kg copper (Cu) and 422 mg/kg iron (Fe), thus these findings show that safflower cake is an excellent source of phosphorus (P), a good source of zinc (Zn) and iron (Fe) (Gowda *et al.*, 2004). Amino acids recorded as percentage protein were 7.8% arginine, 1.8% cysteine, 2.0% histidine, 3.8% isoleucine, 5.5% leucine, 2.9% lysine, 1.4% methionine, 5.2% phenylalanine, 2.9% threonine, 1.2% tryptophan and 4.9% valine (Goss et al., 1956; Gowda *et al.*, 2004). Furthermore, results on decorticated cakes (without hulls) also extracted by an expeller was shown to contain 91.75 % DM, 44 % CP, 14 % CF, 9.7% ether extract, 8.1% ash and 21.2% gross energy, whereas the minerals found were calcium and phosphorus, 0.6 and 11.0%, respectively (Patel, 1966).

Safflower cake has also been found to contain glutamic acid (27.4 g/100 g), aspartic acid (11.14 g/100 g), arginine (8.11 g/100 g), leucine (6.87 g/100 g), proline (5.71 g/100 g), alanine (4.67 g/100 g), valine (4.66 g/100 g), serine (4.57 g/100 g), phenylalanine (3.76 g/100 g) and methionine (1.55 g/100g) (Mariod *et al.*, 2012). Of all the essential amino acids, tryptophan was not found in safflower cake (Mariod *et al.*, 2012). According to Evans and Bandemer (1967) and Rahamatalla *et al.* (1998) the total protein of safflower cake contains high amounts of aspartic acid, glutamic acid, argnine, threonine, leucine, glycine, proline and phenylalanine. The variation in the chemical composition of safflower cake depends on the amount of hulls and on the extent of oil extraction (Heuze *et al.*, 2012).

In view of safflower nutrient composition, safflower seed is usually 106% higher in fat and 21 % lower in CP than whole linted-cottonseed (WLC) (Dschaak *et al.*, 2010). Safflower cake has a much lower content of CP (15.6%) than other cakes such as rapeseed (44%), cottonseed (45%), soybean (47.5%) and sunflower (50.3%), which are used in animal nutrition. The high oil content of safflower seed makes it an attractive energy-dense feed for animals with high energy

requirements, such as lactating dairy cattle (Dschaak *et al.*, 2010). Recently new varieties of safflower seeds are high in oil content and low in fiber than traditional safflower seed varieties (Bergman et al., 2007; Camas *et al.*, 2007; Dschaak *et al.*, 2010). Safflower has the potential to be used as a source of fat for lactating dairy cows. The amino acid composition of safflower cake is close to the composition of soy bean, canola and cotton cake as it was found that the amount of valine, glycine, phenylalanine, methionine and histidine are comparable (Stringhini *et al.*, 2000). The contents of arginine, proline and cysteine in safflower cake were higher than the contents of lysine, leucine, and isoleucine. The content of essential amino acid lysine in safflower cake was two times lower than soybean meal and rapeseed cake, which is a limitation for feed use (Stringhini *et al.*, 2000). Despite its lower crude protein compared to soy bean, cotton seed and sunflower, safflower cake is a good source of protein and can be used as animal feed (Smith, 1996). Safflower cake is similar in quality to palm cake, because it contains much crude fiber (Weiss, 2000). Safflower cake contains fewer minerals than soybean cake. On average the mineral composition of safflower is as follows: Ca (3.6 g/kg), P (8.1 g/kg), K (8.8 g/kg), Mg (3.5 g/kg), Mn (20.4 mg/kg) and iron (122.8mg/kg) while for soy bean cake are Ca (3.8 g/kg), P (7.2 g/kg), K (219 g/kg), Mg (3.1 g/kg), Mn (36.0 mg/kg) and iron (191.0 mg/kg) (Smith,1996). Although safflower cake has fewer minerals, it is a good source of calcium, phosphorus and iron. Safflower cake can be used as a protein supplement in animal feed and partially replacing cotton cake and in this way reduce the bitterness of feed (Weiss, 2000). Comparisons with soybean cake were satisfying only when safflower cake was fed in low amounts (Smith, 1996). Undecorticated safflower cake can be used in ruminant rations as a protein source in replacement of soybean cake, cottonseed cake or linseed cake if used on equal protein basis and if adequate energy is supplied (Kohler *et al.*, 1966). When high levels of safflower cake are included in ruminant diets,

lower performances have been observed (Walker, 2006). Safflower cake is slightly bitter and less palatable than other common ingredients (Smith, 1996). However, its palatability is variable and it may be readily eaten by ruminants when mixed with other feeds (Gohl, 1982).

2.1.2.3 Digestibility

Digestibility of essential amino acids is important as it contributes to the performance of domesticated animals specially pigs and poultry (Kohler *et al.*, 1966). Changes in the concentration of different amino acids was observed in the different safflower cake based the method of processing and the results compared with that of soy bean meal (SBM 44) (Farran *et al.*, 2010). It was shown that the apparent amino acid digestibility (AAAD) of lysine in clean safflower cake (CSC) (81.5%) was lower than that of SBM 44 (86.4%), but higher than that of both ESC (74.7%) and SESC (76.2%) (Farran *et al.*, 2010). However, AAAD of arginine in CSC was 94.2%, being higher than that of SBM (91.2%). Histidine and isoleucine in CSC (87.6%, 85.0%) and SBM (86.2%, 83.9%) respectively, had an AAAD comparable with SESC (84.7%, 81.7%), but higher than that of ESC (81.6%, 80.1%) (Farran *et al.*, 2010). Compared with SBM (87.8%), the AAAD of tryptophan in CSC (84.4%) was similar, whereas that of threonine (81.0% SBM and 74.6% CSC) was lower. The average apparent digestibility of essential amino acids in CSC (85.3%), SESC (82.7%), and SBM 44 (85.2%) was comparable, but only the values of CSC and SBM were higher than that of ESC (80.5%) (Farran *et al.*, 2010).

With respect to true amino acid digestibility (TAAD), the digestibility of lysine (88.3%) in CSC was similar to that of SBM (90.2%), but higher than that of ESC (82.9%) and SESC (84.4%) (Farran *et al.*, 2010). True threonine and tryptophan digestibility in CSC, ESC, and SESC were comparable but lower than those of SBM (Farran *et al.*, 2010). Changes in amino acid concentration were observed among the different SC in response to different processing

methods, especially for CSC. When CSC was compared with SBM 44, the values of TAAD concentrations showed that CSC was higher in arginine (6.63 vs 3.77%), slightly higher in tryptophan (0.57 vs 0.49%), but highly deficient in lysine (1.28 vs. 2.30%) (Farran *et al.*, 2010). The research showed that extensive dehulling of safflower seeds followed by cold extrusion resulted in a low-fiber CSC that is rich in both energy and protein. Compared with SBM 44, this CSC is higher in arginine, slightly richer in tryptophan, but deficient in lysine. In general, the essential amino acid concentration in CSC was slightly higher than that of ESC and SESC. In comparison with the amino acid values of SBM, all dehulled safflower were deficient in lysine but have a much higher arginine level. Similar results on lysine concentration were reported by Valadez *et al.* (1965). Whereas elevated arginine concentration in safflower cake was reported by Kohler *et al.* (1966). Except for the comparable level of lysine, cystine, valine and lower values of tryptophan, all other amino acid concentrations in SESC were higher than those reported for SESC (NRC, 1994).

The digestibility of nutrients in safflower cake was attributed to the seed contents. The seed is 60% hull, constituting of 70% hemicellulose, 21% lignin and 1% ash making it to be less usable to poultry. Even cattle with a built in cellulose digestion vat, the rumen, would be expected to have difficulties with digestion of feeds with high levels of safflower hulls, since it is known that even 5-10% of lignin in alfalfa products greatly hindered the digestion of cellulose and pentosans (Kohler *et al.*, 1966). Safflower cake can be upgraded by reducing the hull content of the seed before extraction. Plant breeding methods and physically removing the hull from the kernel has been used to lower the hull content. These cakes are referred to as partially decorticated cakes. Through plant breeders excellent progress has been made in development of a thin hulled seed with 18-22% hull as compared to 35-40% hull of commercial types. The thin hulled seed have

high protein (35-40%) and low fiber than the usual hull but still contain 20 % lignin. Mechanically dehulled seeds will yield a protein content of 42 % and a fair fiber content of 14-16 % for high energy poultry rations (Kohler *et al.*, 1966). Furthermore, safflower hulls contains about 15-27 % of total digestible nutrients, thus their incorporating in rations reduces feeding efficiency. However, growth is not affected when adequate energy and protein are supplied from other sources (Kohler *et al.*, 1966).

2.1.2.4 Palatability

Safflower cake is not as palatable as compared other feeds such as sunflower, cotton seed and soymeal cakes. However, its palatability can be improved by feeding it in mixed concentrate (Smith, 1996). Unpalatability is due to the two phenolic glucosides matairisenol-β-glucociside and 2-hydroxyarctiin-β-glucoside, which give safflower cake a bitter taste and carthatic properties (Darroch, 1990). These glucosides are allied with protein fraction of the safflower cake. They can be detached by extraction with water or methanol or addition of β-glucosidase (Darroch, 1990). They can also be aloof by combination of physical and enzymatic treatments (Jin *et al.*, 2010).

2.2 Types of safflower livestock feeds

Feeding experiments have been carried out to determine the effects of safflower cake diet as an animal feed. Most researches have shown that safflower cake can be used as a source of protein to most domesticated animals such as poultry, goats, sheep, cattle, dairy and others (Kohler *et al.*, 1966; Dajue and Mündel, 1996; Jacob, 2015). Safflower has been used as range cow cubes and in compounded feeds for cattle and other livestock (Smith, 1996).

2.2.1 Poultry

Due to high fibre content and low energy, safflower cake is of low value to poultry (Kohler *et al.*, 1966). The reduced chick performance reported in the early work of Kratzer and Williams (1951), Petersen *et al.*, (1957), and Kuzmicky and Kohler (1968) was attributed to the relatively high crude fiber, varying between 9 and 16% with different CP levels, in the partially decorticated safflower cake. High dietary fiber is known to hinder chicken performance through reducing the energy content of diets. The average metabolized energy (AME) values of safflower cake determined by Zablan *et al.* (1963) were 1,894 and 1,300 kcal/kg for 2 solvent-extracted meals containing 48 and 23% CP and 14 and 39% crude fiber, respectively. Kohler *et al.* (1966) attributed low energy content of safflower cake to its deficiency in lysine and methionine and its digestibility to amino acids. The use of decorticated safflower cake is possible in poultry if energy level is adjusted, supplementation with lysine and methionine. Recommended inclusion levels are lower for young birds (5-8%) than for older broilers and hens (10-15%) (Darroch, 1990).

Hill and Knowles (1968) reported that safflower cake is a source of dietary mono-unsaturated fatty acid (MUFA) and its inclusion in monogastric diet increases the degree of unsaturated intramuscular fat, without negatively affecting lipid oxidation associated with dietary poly-unsaturated fatty acids (PUFA). Daffa-alla *et al.* (2015) currently reviewed the effect of feeding full fat safflower cake with or without enzymes on the performance and carcass characteristics of the broiler chicks. The results showed that feeding broiler chickens with different levels of safflower cake 0% (sorghum as control), 5, 10, 15 and 20% had no effect on the performance and carcass yield. In all different levels of full fat safflower cake, the chicks gained weight significantly and their feed intake increased. Moreover, addition of commercial enzymes (xylem

500) on different levels of safflower cakes improved the broilers performance. Thus it is economically viable to use full fat safflower cake with or without enzymes as a source of energy in broilers chicks' diet. These results were in line with those recorded by Malakian *et al*. (2011), who found that there is no significant effect on growth performance and carcass trait of broiler chicks when fed safflower cake up to 20% when energy levels and proteins were adjusted. No significant effects were also recorded when laying hens where fed with different levels of safflower seeds (Oguz and Oguz, 2007). However, Cheva *et al*. (1991) reported opposite results. They reported that feeding 15–25% of full fat safflower cake to broiler chicks depressed their weight and feed intake. These findings were in agreement with those of Mohan *et al*. (1984) and Arija *et al*. (1998), who reported that average body weight gain in chickens were decreased by feeding different levels of full fat safflower cake. Adeola and Bedford (2004) and Ghaz *et al*. (2003) attributed the significant improvement in broiler chicken fed with different levels of safflower, supplemented with xylanase enzyme to the fact that exogenous enzyme improved the nutrient utilization and digestibility of energy, fat and protein. These results were also in contrast with those of Makkawi (2009) and Bin-Baraiki (2010) who reported that there are were no significant effects on broiler chickens performance when fed with sorghum and wheat bran, respectively, supplemented with xylanase enzyme. Furthermore, full fat safflower cake is rich in linoleic acid (75-78%) which is important in reducing fat accumulation and promoting muscle growth (Park *et al*., 1997).

2.2.2 Goats

Pinto *et al*. (2011) evaluated the effects of safflower cake dietary supplementary on the growth performance and meat quality of Garganica breed kids. The results showed that supplementation with dietary safflower cake (20%) did not induce significant changes in the final body weight

and average daily gains of kids (Pinto *et al.*, 2011). On the other hand, a lower feed intake was observed in safflower cake group in comparison with the control group. Feed intake for the kids supplemented with safflower was 0.621 kg/ day while that of the control was 0.765 kg/day (Pinto *et al.*, 2011). As a consequence, the kids fed with safflower cake group showed a better feed conversion ratio (3.69) compared to that of the control group (4.24). Moreover, Pinto *et al.* (2011) found that the use of safflower cake decreased the level of saturated fatty acid (SFA) and increased the level of monounsaturated fatty acid (MUFA) in kid meat. In particular, safflower cake dietary supplementation decreased the level of saturated fatty acids (38.65% against 44.73% for control, $P < 0.01$) and increased the level of monounsaturated fatty acid (54.54% against 47.82% control, $P < 0.01$) in the kid meat. The control group showed a higher level of polyunsaturated fatty acids when compared to safflower cake group (7.43 % versus 6, and 80 %), even if the unsaturated fatty acids level was lower in *Longissimus lumborum* muscle of kids fed control diet (55.26 % versus 61.34%, $P < 0.01$). As lipid was extracted from the animals, kids from the control group contained more omega-6 (ω-6) fatty acids in comparison to kids feeding experimental diet (6.61 % versus 5.74 %, respectively) while the opposite trend was observed for the level of omega-3 (ω- 3) fatty acids (1.06 % versus 0.82 %). In this study, the ω-6 to ω-3 ratio was significantly affected by diet and in particular this ratio decreased in meat of kids fed experimental diet (5.55 against 8.05 for safflower cake and control group, respectively, $P < 0.05$) being closer to the value suggested by the Italian Society of Human Nutrition which recommend a ω-6 to ω-3 ratio of 4 (Reiser and Shorland, 1990). These researches showed that the use of safflower cake in kid's nutrition does not have any significant effect on live weight and average daily gain even if kids were fed with safflower cake. The safflower diets had a lower feed intake and a better feed conversion ratio. The use of safflower increased the level of unsaturated fatty

acid (UFA) in kid meat. The improvement of the intramuscular fat quality (α-6 to α-3 ratio, unsaturated to saturated ratio) may be a very important target for meat production in order to protect the consumer health (Pinto *et al.*, 2011).

Pinto *et al.* (2011) and Ragni *et al.* (2015) recently carried out an experiment also based on the effect of dietary safflower cake on growth performance, carcass composition and meat quality traits in Garganica breed kids. The kids were fed with soy bean meal as a control and soy bean meal supplemented with 20% safflower as the experimental diet. Findings of the performance trial of kids showed that no dietary effect was observed neither for the live body weight nor for the kids' growth traits. The initial live body weight were 11.95 control and 12.07 kg safflower diet and their final live body weights were 21.0 and 20.3 kg, respectively. However, kids fed with the soybean diet reported a higher feed consumption (0.76 kg/day against 0.62 kg/day), whereas when fed safflower cake kids showed a better feed efficiency (5.15 kg/day versus 3.91 kg/day), thus feed conversion ratio was enhanced by dietary safflower cake. Analyzing the *longissimus lumborum* meat chemical composition showed that the soy bean diet contained 75.81% moisture, 19.72% protein, 3.17% lipid and 1.08 % ash content, while safflower fed contained 74.80%, 18.98 %, 3.73% and 1.00%, respectively, for the same muscle. The chemical composition of meat did not differ between groups and the results were similar to those reported by Caputi *et al.* (2007). The study confirmed that safflower cake can be used in kids/lamb total mixed rations (TMR) diets as it had no significant reduction of productive performance and meat quality. Its use may be considered as a sustainable and economically viable strategy because of the lower cost of the safflower by product (Ragni *et al.*, 2015).

2.2.3 Sheep

Inclusion of safflower cake in total mixed rations for growing and fattening small ruminants does not affect their growth performance, meat quality or lipid fatty acid profile (National Research Council (NRC), 2007). Safflower cake successfully replaced corn, soy bean and wheat bran meal, when they were formulated to contain 180 g/kg CP and 9.4 MJ/kg DM of metabolizable energy (NRC, 2007). Supplementation with safflower cake resulted in a greater final weight of lambs (23.9 kg) compared to the control (21.0 kg) (NRC, 2007). ZoBell *et al.* (2005) had earlier reported that safflower by products can be an effective replacement in concentrate with no adverse effect on livestock performance compared to control diets. Furthermore, safflower cake significantly improves feed to gain ratio in lambs (Tufarelli *et al.*, 2013). Tufarelli *et al.* (2013) reported that the feed to gain ratio was 4.5. Their results were in agreement with other studies reporting that feed conversion ratio is associated with higher weight gain (Nagalakshmi *et al.*, 2011). Moreover, safflower cake in TMR does not affect values of meat moisture, protein, lipid and ash in lambs (Tufarelli *et al.*, 2013). Tufarelli *et al.* (2013) recorded the values for meat moisture, protein, lipid and ash as 75.78%, 19%, 3.27%, and 1.24%, respectively. The results were in accordance with those reported by Ravi *et al.* (2000) using other oilseed by-products in concentrate for lambs and by Srivastava *et al.* (1990), who evaluated the addition of different oilseed cakes in kid rations. Safflower cake also affected meat fatty acid profiles in lambs (Tufarelli *et al.*, 2013). The saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and ω-6 / ω-3 were decreased in alternate safflower diet. These ratios were similar to those found by Vicenti *et al.* (2009) in the *Longissimus dorsi* muscle of other species when fed different seed meals in the diet. Safflower cake also increased UFA / SFA ratio in the meat of lambs. These ratios are important since they express the nutritional value of lipid for human health (Peiretti

and Meineri, 2008). The use of safflower cake in TMR also improves the atherogenic and thrombogenic indexes of the *Longis-simus dorsi* muscle of lambs which is functional product recommended in healthy balanced dietary to limit human cardiovascular diseases, as also reported in meat of rabbits (Peiretti and Meineri, 2008), bulls (Vicenti *et al.*, 2009) and poultry (Laudadio and Tufarelli, 2010).

2.2.4 Dairy cattle

Safflower cake is also a valuable ingredient for dairy cows, with no noticeable effect on flavour or odour of milk produced (Smith, 1996). Safflower cake can be a good substitute to linseed meal for dairy cattle (Smith, 1996). Adding 1 kg of safflower cake in the rations of Lithuanian dairy cows increased milk yield and milk fat by 1.4% and 0.37%, respectively. The substitution of 1 kg of concentrate by similar amount of safflower cake gave similar results (Juknevicius *et al.*, 2005). With low-producing Friesian cows producing 10 kg milk/day, 3.75 kg of safflower cake successfully replaced 3 kg of undecorticated cottonseed oil-meal, slightly increasing milk fat content (El-Shinnawy *et al.*, 1979).

There has been a resurgence of interest in feeding supplemental fat to cattle because of the benefits that dietary fat can exert on reproduction (Staples *et al.*, 1998) and on the quality of ruminant-derived food products (Bauman *et al.*, 2000). Ruminant milk fat has several unique compositional characteristics, one of which is conjugated linoleic acid (CLA) (Palmquist and Schanbacher, 1991). Certain isomers of CLA are known to regulate fatty acid metabolism in various tissues including the mammary gland. Baumgard *et al.* (2002), demonstrated that *trans*-10, *cis*-12 CLA isomer altered expression of genes involved in milk lipid synthesis in dairy cows by decreasing mRNA for acetyl-CoA carboxylase, fatty cid synthase, and stearoyl-CoA desaturase. Lipogenic enzymes are regulated, in part, through mRNA transcription (Munday,

2002; Stoeckman and Towle, 2002; Barber *et al.*, 2003); thus, quantifying the respective mRNA should provide insight into their dietary regulation. Feeding oilseeds to lactating dairy cows is one method to change the proportion of unsaturated fatty acids (UFA) in milk fat with increases as high as 40% (Casper *et al.*, 1990; Stegeman *et al.*, 1992; Kim *et al.*, 1993), although extensive biohydrogenation (BH) normally occurs in the rumen (Palmquist and Jenkins, 1990). Safflower seed have a beneficial effect on human health, as safflower seed is rich in polyunsaturated fatty acids (PUFA), and a source of linoleic acid (0.76 of total FA). Bell *et al.* (2006) reported that addition of safflower oil at 60 g/kg dry matter increased cis-9, trans-11 CLA in milk, which has been suggested to be the best natural source of CLA in the human diet due to its anticarcinogenic properties (Pariza and Hargraves, 1985). Diet-induced changes in ruminal BH with enhanced levels of CLA in milk fat are also associated with a decrease in milk fat content. Under certain dietary conditions, the pathways of ruminal BH are altered to produce unique FA intermediates (Bauman and Griinari, 2001), some have been identified as potent inhibitors of milk fat synthesis in the mammary gland of dairy cows, such as *trans*-10, *cis*-12 CLA (Baumgard *et al.*, 2000). Feeding safflower oil to lactating dairy cows increased levels of *trans*-10, *cis*-12 CLA in milk fat (Bell *et al.*, 2006), similar to studies involving diet-induced milk fat depression (Piperova *et al.*, 2000; Peterson *et al.*, 2003). Dschaak *et al.* (2010) did a lactation study to assess productive performance of dairy (Holstein) cows fed to varying levels of whole safflower seed and identify its impact on milk fat content and milk FA composition. The whole safflower seed was added to diets by replacing whole linted-cottonseed (CS) in the CS diet. Intake of dry matter (DM), nitrogen (N) and fibre decreased by the whole safflower seed diet compared to the CS diet (Dschaak *et al.*, 2010). Increasing the level of whole safflower seed (WSS) inclusion linearly decreased N intake, but linearly increased fiber intake (Dschaak *et al.*, 2010). Digestibilities of

DM and organic matter (OM) increased in the WSS diet compared to the CS diet, whereas they linearly decreased with increasing dietary level of WSS. Milk yield averaged 33.7 kg/day, and was similar among dietary treatments (Dschaak *et al.*, 2010). Milk fat content linearly decreased with increasing WSS inclusion, while milk true protein and lactose contents did not differ among dietary treatments (Dschaak *et al.*, 2010). Milk fat content was affected when WSS was included at 40 g/kg DM, with 11% reduction (Dschaak *et al.*, 2010). Efficiency of use of feed N to milk N also increased, but milk urea N decreased by feeding the WSS diet compared to the CS diet, implying that WSS supplementation improved dietary N use for milk production (Dschaak *et al.*, 2010). *Cis*-9, *trans*-11 CLA, and *trans*-10 18:1 FA linearly increased with increasing WSS inclusion level (Dschaak *et al.*, 2010). The study of Dschaak et al. (2010) showed that supplementing WSS in diary diets can be a promising means of fat supplementation to lactating dairy cows without negative impacts on lactational performance at up to 30 g/kg DM. The increased *cis*-9, *trans*-11 CLA concentration due to addition of WSS can enhance milk quality because of its potentially beneficial health effects (Dschaak *et al.*, 2010). However, the beneficial effect of WSS was counterbalanced by an unfavourable increase of *trans*-10 18:1 FA (Dschaak *et al.*, 2010). Bottger *et al.* (2002) reported that lactating beef cows fed high-linoleate safflower seeds were more capable of maintaining body condition, whereas cows fed high-linoleate safflower seeds had greater total milk fat. Cattle fed supplemental oilseeds have greater concentrations of CLA in meat (Wood *et al.*, 1999) and milk (Griinari and Bauman, 1999), which may constitute a health advantange to consumers of these food products (Bauman *et al.*, 2000).

2.5.5 Beef cattle

For beef cattle safflower cake has been reported to increase weight when it is mixed with other oil meals to improve its palatability. Voicu *et al.* (2009) reported in an experiment with steers having the initial body weight of 285 kg. The steers were divided into 3 groups and fed safflower cake mixed with wheat silage. The first group was the control (fed with wheat silage only), second group (fed with wheat silage + 180 g/kg safflower seed) and third group (fed with wheat silage + 350 g/kg safflower seed), respectively. The results showed that inclusion of safflower cake in wheat silage had no adverse effect on feed intake and feed palatability, and resulted in an average daily gain higher than 1.4 kg/d (Voicu et al., 2009). Encinias *et al.* (2001) investigated how safflower supplementation affected breeding cow performance and the growth of calves. In this experiment, cross bred cows with initial weight of 601.4 kg were fed similar diet containing 51 g/kg dietary fat 45 days prior to calving. Rolled safflower diet containing 320 g/kg ether extract and 800 g/ kg linoleic acid in higher fat diet, while safflower cake was included in low fat diet. At the end of the experiment, cows fed with low fat diet (safflower cake) scored higher body weight than those fed higher fat diet (Encinias *et al.*, 2001). The birth weights of calves and weaning weights were similar for both treatments. Although the final body weights were similar in the two treatments, the cows fed with higher fat diet showed a higher feed intake. Thus supplementation of breeding cows with safflower seeds did not improve cows nor calves performance as the safflower cake improved the cow's performance, though it did not improve the calf performance (Encinias *et al.*, 2001). In another study carried out by Schooljegerdes *et al.* (2009) investigated whether lipids contained in safflower cake have a beneficial effect in the reproduction of beef cows. Initially, cross bred cows with live weight of 411kg were fed foxtail millet hay starting one day postpartum at 1.68% of body weight (DM basis) or a low fat

concentrate containing 637 g cracked maize, 334 g safflower cake and 29 g liquid molasses, or high linoleate concentrate (953 g cracked high linoleate (790g/kg 18:2n-6 fatty acid) safflower seeds and 47 liquid molasses, on DM basis, as control. The results showed that the treatment diet did not influence the ovarian follicular development, pypophyseal concentration of the luteinizing hormone (LH) and concentration of Insulin-like growth factor (IGF-1) of the liver (Schooljegerdes *et al.*, 2009). In contrast, anterior pituitary glands from linoleate cows contained more follicle stimulating hormone (FSH) than the control cows (Schooljegerdes *et al.*, 2009). Linoleate cows had less Insulin-like growth factor (IGF-1) in the medial basal hypothalamus. In conclusion, fat supplementation with high-linoleate safflower seeds did not improve the development of ovarian follicles and detrimentally affected early postpartum fertility possibly because of a reduction in IGF-I concentrations in tissues essential to reproduction (Schooljegerdes *et al.*, 2009).

2.5.6 Pig

Undecorticated safflower cake is not suitable for pig feed as its protein quality is low, due to its deficiency in essential amino acids such as methionine, lysine and isoleucine, and excessive fiber content (Darroch, 1990). Similarly as in other animal feeds, decortication improves the nutritional value of the safflower cake for pigs. Decorticated safflower cake can be used as meal for growing pigs provided it is supplemented with a protein source high in the amino acid lysine. Up to 12% of safflower cake could be included in the diet if lysine requirement is met (Darroch, 1990). According to Chiba (2001), safflower cake should not provide more than 5 to10 % or 12.5% of the supplemental protein in the diet. These are in agreement with the study by Williams and O'Rourke (1974), who reported that feeding growing pigs (20 to 80 kg) with a diet containing 17% of decorticated safflower cake reduced growth performance and increased

carcass fatness. Moreover, results showed that addition of lysine (0.2%) resulted in a small increase in performance but results were still lower than those obtained with 13% soybean meal or 9% fish meal supplements (Williams and O'Rourke, 1974). Finisher female pigs fed safflower cake diets supplemented with amino acids had reduced growth, and a small increase was observed when the safflower cake diet was supplemented with fish meal instead of lysine and methionine (Williams and O'Rourke, 1974). However, when safflower cake was fed at 14.5% supplemented with 0.2% lysine, 0.1% methionine and 4.5% fishmeal fed to 46 to 87 kg barrows and gilts, an improvement was observed in average daily gain and a slight fat carcass, 65 and 27%, respectively. It was also found that barrows grew faster and produced fatter carcasses than gilts, but a significant sex to diet interaction occurred which made gilts to be concluded to be better fed ad-libitum without detriment to the quality of the carcass (Williams and O'Rourke, 2012). Dehulled safflower cake can be included up to 15% in the diet of pregnant sows. Feeding safflower cake to weanling pigs or pigs weighing less than 45 kg is not recommended (Chiba, 2001).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out in The Botswana University of Agriculture and Natural Resources (BUAN), Notwane Farm, which is 24° 35'S and 25°58'E: 998 m above the sea level. The climate is semi-arid with average annual rainfall of 538 mm. Most rains falls in summer, which generally starts in the late October and continues to March or April. During the rainy seasons, prolonged dry spells are common and rainfall trends are localized. The soils are deficient in phosphorus, have low levels of nitrogen and organic matter (Emongor *et al.*, 2004; Emongor *et al.*, 2012). The soils are shallow, ferruginous tropical soils, mainly consisting of medium to coarse grains and sandy loams with low water holding capacity and subject to crusting after heavy rains (De Wilt and Nachtengale, 1996). The experimental site has an average maximum and minimum temperature varying between 33.1–34.7 °C and 19.2–19.5 °C, respectively (Ramolemana, 1999).

3.2 Experimental design

The experimental design was a Randomized Complete Block Design (RCBD) with nine treatments replicated three times. The experiment was blocked because there was a gentle slope of about 1% in the experimental site. The treatments were nine genotypes of safflower Kiama Composite (control), PI 537632-1038-USA, PI 3044-BJ-2621-Iran, PI 537598-Sina-USA, PI 407616-BJ-2131-Turkey, PI 537634-1040-USA, PI 537668-BJ-1085-USA, PI 314650-Milutin-114-Kazakistan, and PI 306830-BJ-1632-India). Safflower was planted at a spacing of 45 cm x

20 cm. The treatments were randomized within the experimental blocks. The seeds were planted in single row spacing at a depth of 2.5 cm.

3.3 Crop husbandry

The field was ploughed using a mouldboard plough and a disc harrow was used to prepare a fine tilth. The soil samples were taken and analysed to know which fertilizers to apply. Weeding was done by hoeing between rows to control weeds because safflower at rosette stage is a poor competitor (Dajue and Mundel, 1996). Other management practices such as irrigation and pest control was carried out when necessary. The moisture content of the experimental plots was maintained at field capacity.

3.4 Data collection

The dependent variables measured were dry matter, whole seed oil content, crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ash, mineral analysis of leaves, whole seed and cake after oil extraction.

3.4.1 Dry matter determination (%)

The dry matter content was determined by drying weighed (20g) safflower leaves, whole seed and cake at 66 °C for 72 hours. After drying, the samples (leaves, whole seed and cake) were re-weighed and their dry matter content was determined by subtracting the dry weight from their corresponding fresh weights, respectively (AOAC, 2000). The dry matter was expressed as:

% dry matter (DM) = sample of dry weight/sample of fresh weight *100

3.4.2 Whole seed oil content

Safflower oil was extracted by pressing safflower whole seeds using an electric oil expeller (Oil Love, National ENG CO.LTD). The oil expeller was preheated to a temperature of 180–200 °C for twenty minutes. Safflower whole seeds (1 kg) were used for oil extraction. The oil yield was determined by weighing the oil expressed. The oil yield was expressed as a percentage of the whole seed that was used for expressing the oil.

3.4.3 Crude protein determination

Crude protein was determined using the Kjeldahl digestion method (AOAC, 2000). The samples (0.5g) were digested in 10 ml of concentrated sulphuric acid (98%) and 2 ml of 15–45% hydrogen peroxide in a digestion block heater for 8 hours, kept at 420 °C. After digestion, the samples were allowed to cool for 2 hours and transferred into 200 ml volumetric flasks and filled with distilled water to the mark. The digested samples (0.5 g) were titrated with 0.1142 N sulphuric acid until colour turned purple. The amount of acid used to titrate will gave an indication of the amount of nitrogen present in the safflower leaves, whole seed and cake. The protein was estimated by multiplying the nitrogen (N) content by 6.25 (AOAC, 2000).

3.4.4 Determination of neutral detergent fiber (NDF)

This is the insoluble residual material consisting of major cell wall constituents such as cellulose, lignin and hemicelluloses. Analysis for NDF entailed first boiling 0.5g of safflower samples (leaves, whole seed and cake) for one hour in 2000 ml neutral solution of sodium laurel sulphate and ethylene dimethyl tetra acid (EDTA). The detergent removed lipids, sugars, organic acids and other water soluble materials: pectins and non- protein nitrogenous compounds and water

soluble proteins. The material remaining after boiling for one hour was the Neutral Detergent Fiber (NDF) (AOAC, 2000).

3.4.5 Determination of acid detergent fiber (ADF)

This is the indigestible residues of cutin silica and some pectins. Components of feed which are insoluble in neutral detergent are readily dissolved in an acid detergent and extracted. Firstly, the sample residues were weighed to approximately 1 g into a tared refluxing baker. Acetyl ammonium bromide (100 g) was dissolved into a 0.5 M sulphuric acid (an acid detergent solution) and 2 ml of decahydronaphthalene. The mixture was heated rapidly to make it boil in about 5-10 minutes and the heat was lowered to reduce the foams as it boils. This was continued for one hour from the onset of boiling and then boiling was adjusted to a minimum uniform level. After boiling for additional 5-10 minutes, the heat was turned off and the mixture was filtered onto a tared crucible (Wc) with a gentle suction, the residue was washed twice with hot water (90-100 °C). The sides of the crucible were also rinsed with hot water. The residue was then rinsed with acetone until the filtrate became clear and then oven dried at 105 °C overnight, cooled and weighed. The residue was the ADF (AOAC, 2000).

3.4.6 Determination of acid detergent lignin (ADL)

This is the residue material consisting of acid insoluble ash, cutin and traces of silica. This is an adaptation of Van Soest detergent scheme which uses the ADF method. All the protein and other safflower leaf, whole seed and cake residue components which dissolve in an acid medium were removed by the detergent (Goering and Van Soest, 1970). After completion of ADF analysis, the cooled filter bags (containing the bag and sample weight) are returned into the ANKOM Daisy II Incubator vessel (3.7 L) with 40 ml sulphuric acid (72%) the same day. The samples were

agitated and heated for one hour after which the detergent solution was removed from the digestion vessels. The filter bags were rinsed three times with 2000 ml of hot water (90–100 °C) while still on the incubation vessels. After rinsing, they were removed and placed in 250 ml beaker and soaked for three minutes in acetone to remove water. The filter bags with samples were spread to allow acetone to evaporate and oven dried at 105 °C overnight. After removal from the oven, there were cooled to ambient temperature and weighed (Goering and Van Soest, 1970). Percentage ADL was calculated as;

$$\% \text{ ADL} = [W3 - (W1 \times C1)/W2] \times 100$$

Where: W1 = Bag weight, W2 = Sample weight, W3 = Dried weight of bag with fiber after extraction process and C1 = Blank bag correction (final oven–dried weight divided by original blank bag weight).

3.4.7 Ash determination

This is the non-volatile inorganic residue remaining after the combustion of an organic material. The amount of ash in the samples (leaves, whole seed and cake) were ascertained by completely burning to ash samples previously used in ADL procedure (ANKOM method) in a muffle furnance of 550- 600 °C for 2 hours. During burning, water, protein, fat and carbohydrates were completely removed (AOAC, 2000). The percentage ash content was expressed by the following equation;

% Ash= weight of burnt samples/ weight before burning * 100

3.4.8 Mineral analyses

Safflower samples (leaves, whole seed and cake) dried at 66 °C for 72 hours were used for mineral analyses. The dried samples were ground in a mill to pass a 1 mm sieve screen. Then 0.5 g of dried samples were digested in sulphuric as described in 3.4.3 above. The samples (0.5 g) were then to be used for the different analysis of minerals. Mineral analysis (P, K, Ca, Mg, Na, Fe, Zn, Mn and Cu) were determined using inductively coupled plasma mass spectrometry (ICP-MS) machine which measures the concentration of elements in a sample (AOAC, 2000).

3.5 Data analysis

Data collected was subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2011). Where a significant F- test was observed, treatment means were separated using the Least Significant Difference (LSD) at $P = 0.05$.

CHAPTER 4

4.0 RESULTS

4.1 Leaf components

4.1.1 Dry matter

The results of the study showed that genotype and growing season significantly ($P < 0.05$) influenced safflower leaf DM contents (Figure 1). The leaf DM contents ranged between 88.1–91.2% and 88.8–90.8% in winter and summer grown safflower, respectively (Figure 1). The leaf DM (93.4%) of summer grown safflower was significantly ($P < 0.05$) higher than the leaf DM (89.0%) of winter grown safflower (Figure 1). In winter, the genotype PI 537668-BJ-1085-USA (91.2% DM) had significantly ($P < 0.05$) higher leaf DM than the genotype PI 537632-1038-USA with 88.1% DM (Figure 1). However, the other genotypes did not significantly ($P > 0.05$) differ in their leaf DM in winter grown safflower (Figure 1). In summer grown safflower, the genotypes did not significantly ($P > 0.05$) differ in their leaf DM content (Figure 1).

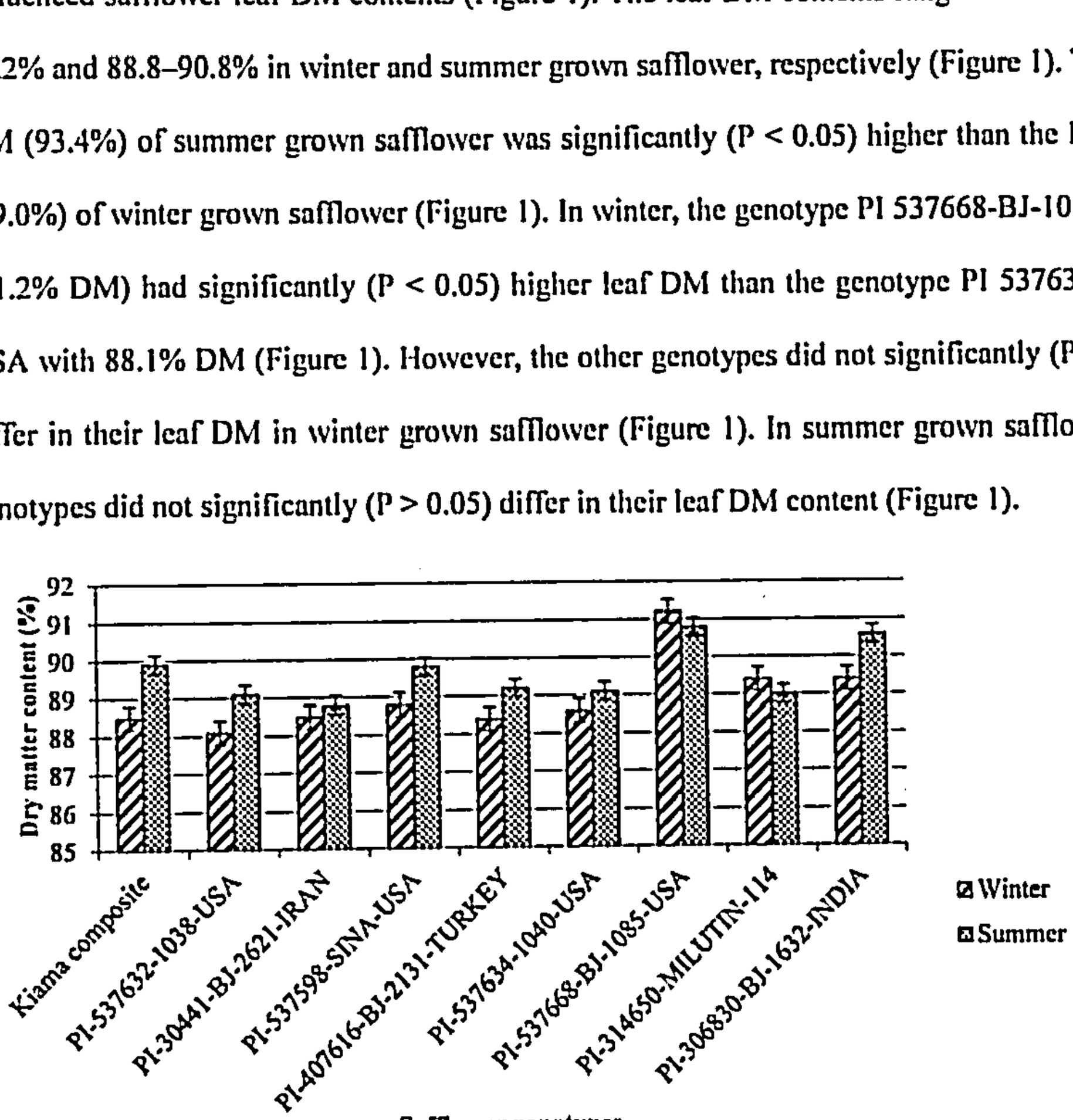

Figure 1. Effect of genotpe and growing season on safflower leaf dry matter

4.1.2 Crude protein content

Leaf CP was significantly ($P < 0.05$) influenced by safflower genotypes and growing season (Figure 2). The leaf CP contents ranged between 22.1–27.7% and 23.4–27.5% in winter and summer grown safflower, respectively, depending on genotype (Figure 2). In winter, the genotype PI 537634-1040-USA had significantly ($P < 0.05$) higher leaf CP (27.7%) content than the genotypes Kiama composite and PI 30441-BJ-2621-Iran which all had leaf CP content of 22.1% (Figure 2). However, the genotypes PI 537634-1040-USA, PI 314650-Milutin-114-Kazakistan, PI 537632-1038-USA, PI 407616-BJ-2131-Turkey, PI-306830-BJ-1632-India and PI 537668-BJ-1085-USA did not significantly ($P > 0.05$) differ in their leaf CP contents in winter (Figure 2). In summer, the genotypes PI-537634-1040-USA (27.5% CP), PI 314650-Milutin-114-Kazakistan (27.2% CP) and PI 407616-BJ-2131-Turkey (26.5% CP) had statistically similar leaf CP content, but had significantly ($P < 0.0001$) higher leaf CP than the genotypes Kiama composite, PI 537632-1038-USA, PI 30441-BJ-2621-Iran, PI 537598-Sina-USA, PI 537668-BJ-1085-USA and PI 306830-1632-India (Figure 2). The genotype Kiama composite had significantly ($P < 0.0001$) lower leaf CP (22.1%) than all the other genotypes under study (Figure 2). The genotypes PI 537598-Sina-USA, PI 537668-BJ-1085-USA and PI 306830-1632-India did not differ statistically in their leaf CP contents, but had significantly ($P < 0.0001$) higher leaf CP contents than the genotypes PI 537632-1038-USA and PI 30441-BJ-2621-Iran (Figure 2).

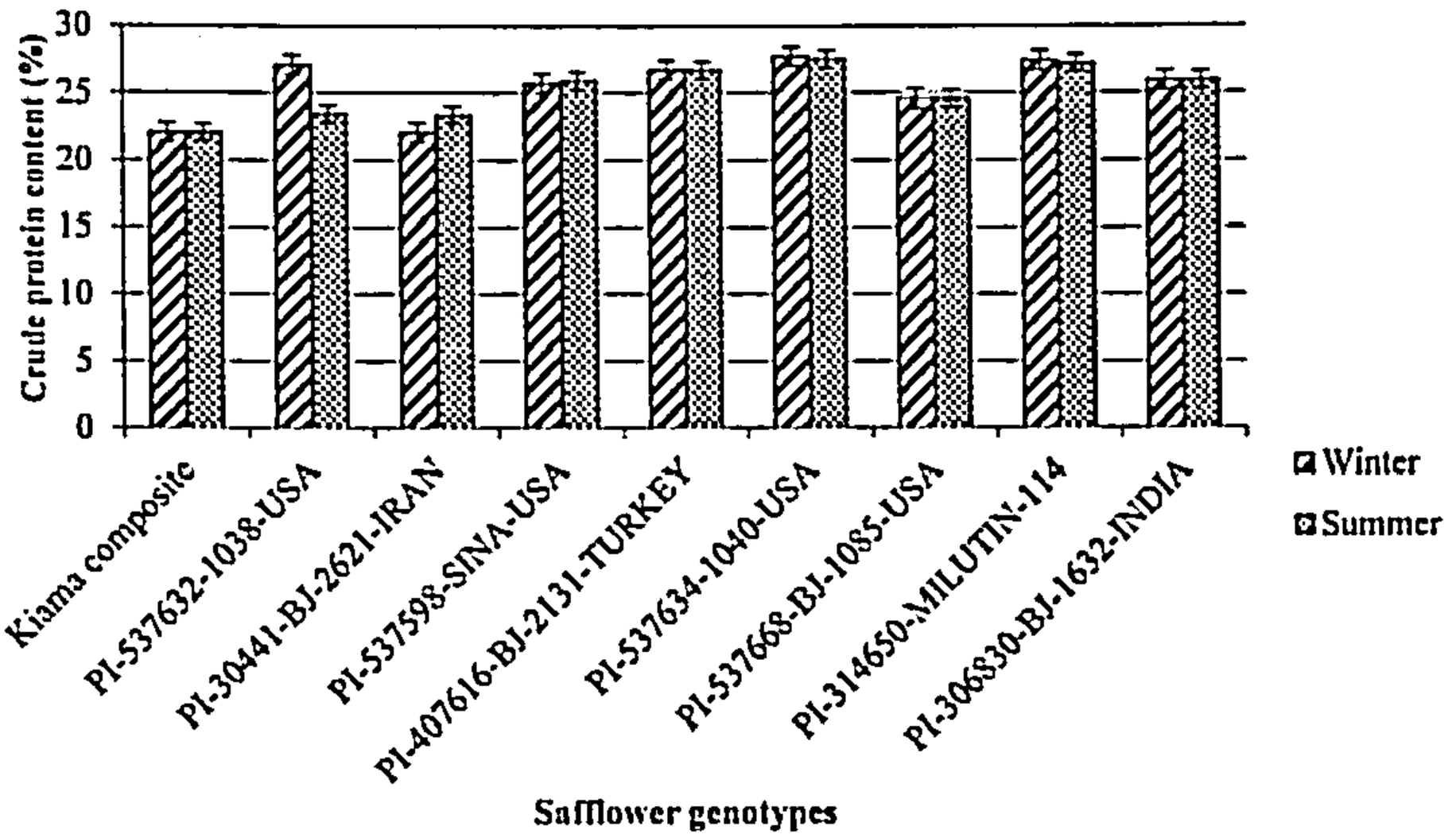

Figure 2. Effect of genotype and growing season on safflower leaf crude protein

4.1.3 Neutral detergent fiber

The leaf NDF significantly ($P < 0.05$) differed depending on safflower genotype and growing season (Figure 3). The variation in leaf NDF ranged between 20.5–26.2% and 21.9–25.3% in winter and summer, respectively (Figure 3). On average, most genotypes had high leaf NDF in winter than in summer (Figure 3). In summer grown safflower, the genotype PI 314650-Milutin-114-Kazakistan had the highest leaf NDF of 26.2% which was significantly ($P < 0.05$) different from the leaf NDF of the genotype PI 407616-BJ-2131-Turkey (20.5%), but was not significantly ($P > 0.05$) from that of the other genotypes (Figure 3). Also the genotypes Kiama composite and PI 537634-1040-USA had significantly ($P < 0.05$) higher leaf NDF than that of the genotype PI 407616-BJ-2131-Turkey (Figure 3). PI 407616-BJ-2131-Turkey grown in summer had the highest leaf NDF (25.3%) and was not significantly ($P > 0.05$) different from that of all other safflower genotypes (Figure 3).

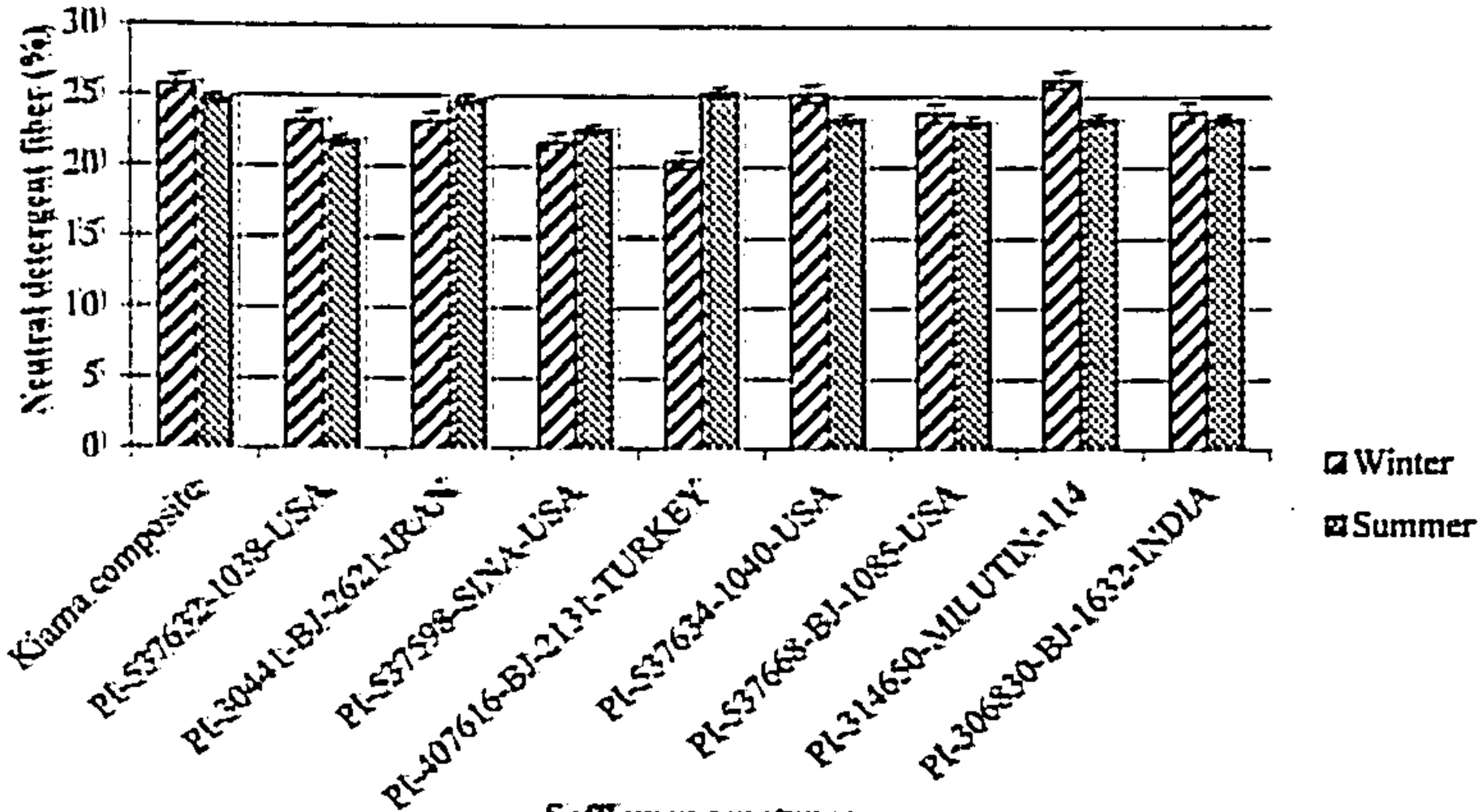

Figure 3. Effect of genotype and growing season on safflower NDF

4.1.4 Acid detergent fiber

Safflower genotype and growing season did not significantly ($P > 0.05$) influence the leaf ADF (Figure 4). The leaf ADF varied between 26.5–32.7% and 30.0–32.0% in winter and summer, respectively (Figure 4).

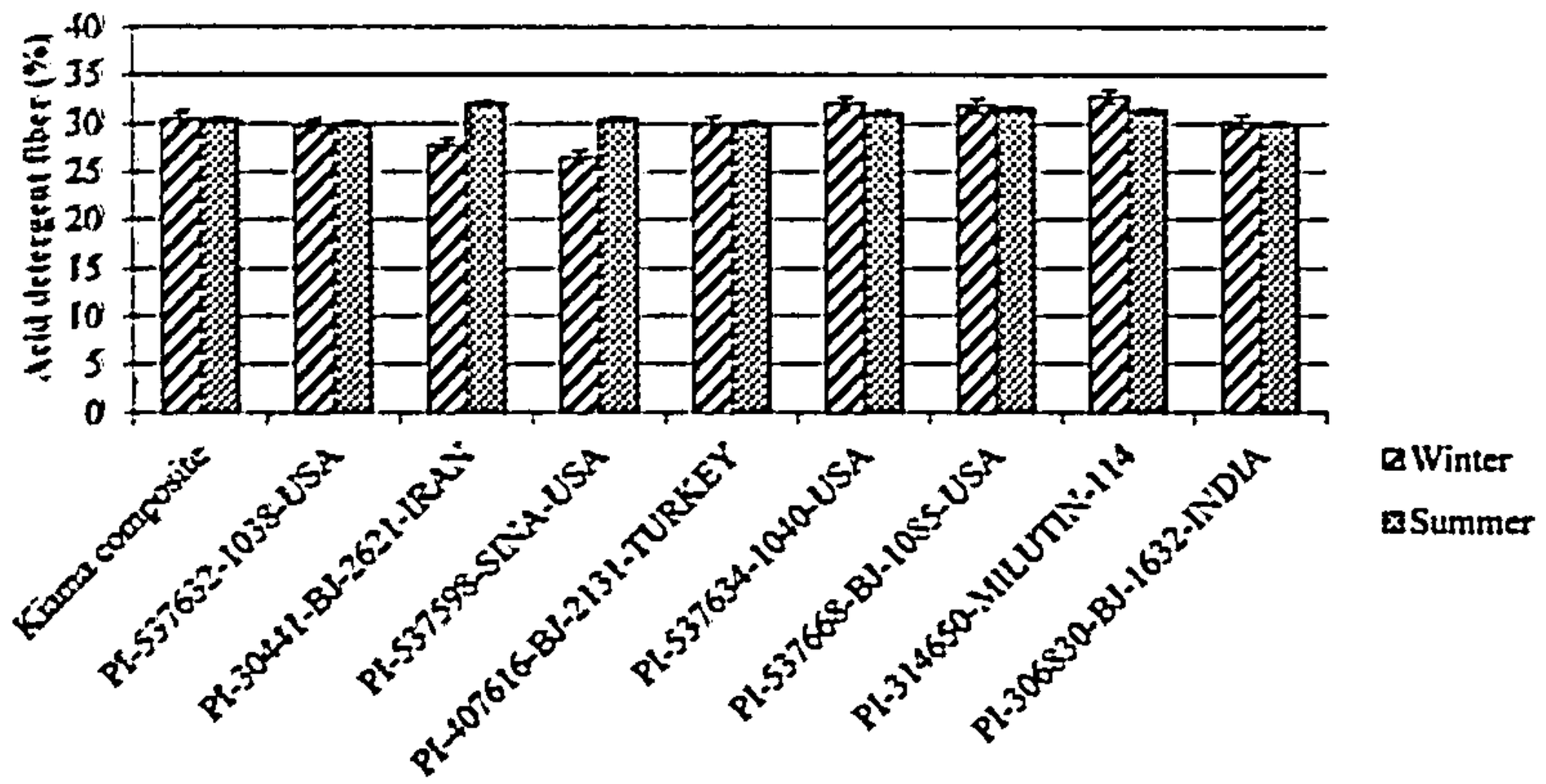

Figure 4. Effect of genotype and growing season on safflower leaf ADF

4.1.5 Acid detergent lignin

There were significant ($P < 0.05$) differences in leaf ADL depending on safflower genotype and growing season (Figure 5). The leaf ADL content ranged between 6.7–10.7% and 7.3–9.0% in winter and summer, respectively (Figure 5). In winter grown safflower, PI 407616-BJ-2131-Turkey had leaf ADL of 10.7% which was significantly ($P < 0.05$) higher than the leaf ADL content of the genotypes PI 30441-BJ-2621-Iran, PI-314650-Milutin-114-Kazakistan, PI 537632-1038-USA, PI 537598-Sina-USA and PI 306830-BJ-1632-India (Figure 5). The leaf ADL of the genotypes PI 537668-BJ-1085-USA, Kiama composite and PI 537634-1040-USA did not significantly ($P > 0.05$) differ than that of the genotype PI 407616-BJ-2131-Turkey in winter grown safflower (Figure 5). Also in summer grown safflower, the leaf ADL content of the genotype PI 537634-1040-USA was significantly ($P < 0.05$) higher than that of the genotypes PI-314650-Milutin-114-Kazakistan and PI 537632-1038-USA (Figure 5). However, in summer grown safflower, the leaf ADL contents did not significantly ($P > 0.05$, LSD = 2.00) differ within genotypes (Figure 5).

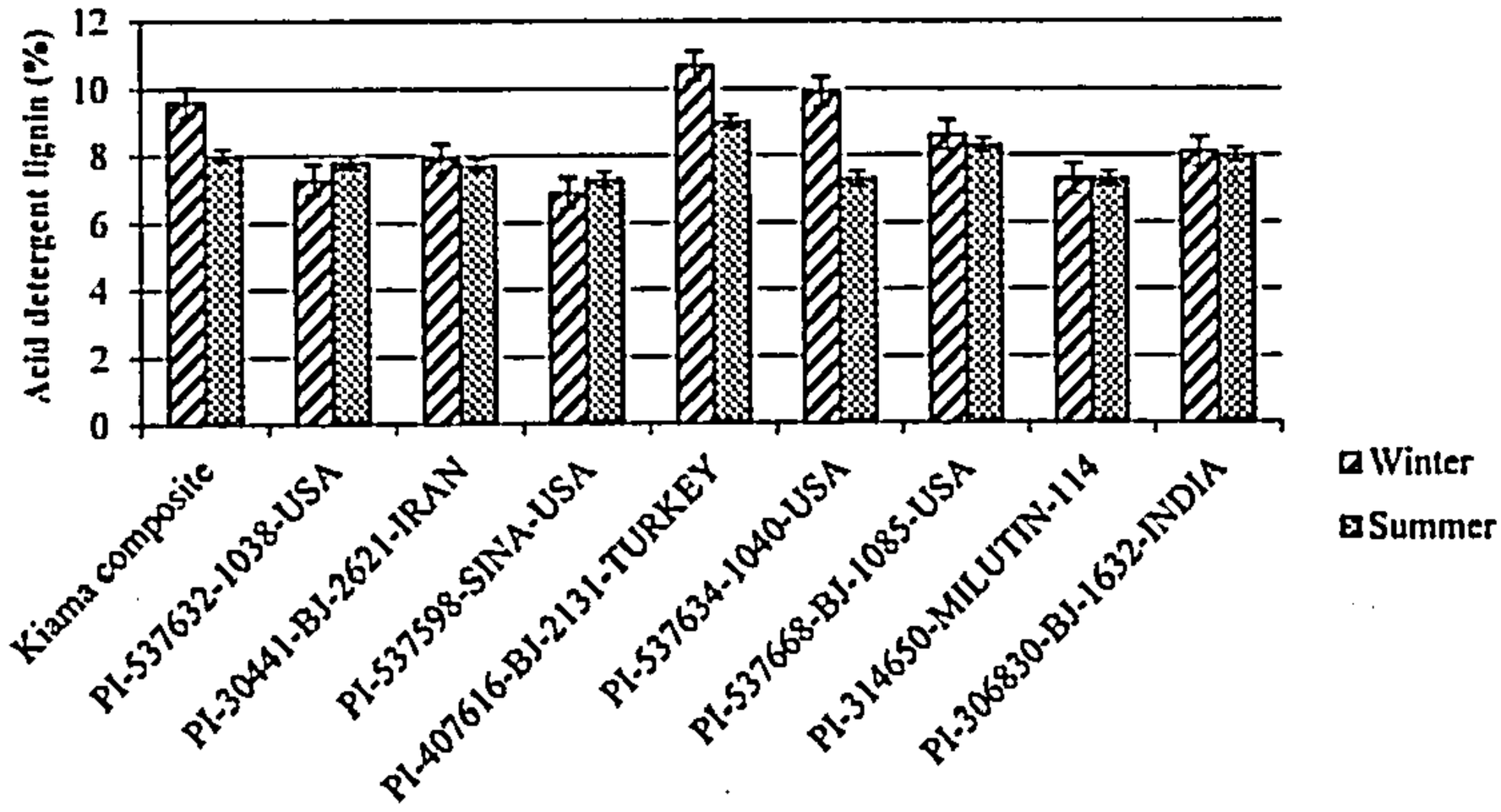

Figure 5. Effect of genotype and growing season on safflower leaf ADL

4.1.6 Ash content

Safflower genotypes and growing season also significantly ($P < 0.05$) influenced the leaf ash contents (Figure 6). The leaf ash contents ranged between 0.83–1.08% and 0.90–1.13% in winter and summer, respectively, depending on genotype (Figure 6). In winter grown safflower, the genotype PI 537632-1038-USA with the highest ash content of 1.08% had significantly ($P < 0.05$) higher leaf ash content than the genotypes PI 314650-Milutin-114-Kazakistan, PI 537668-BJ-1085-USA and PI 537598-Sina-USA (Figure 6). However, the leaf ash content of the genotype PI 537632-1038-USA was not statistically ($P > 0.05$, LSD = 0.15) different from PI 407616-BJ-2131-Turkey, PI 306830-BJ-1632-India and Kiama composite (Figure 6). Whereas in summer grown safflower, the leaf ash content of the genotype PI 30441-BJ-2621-Iran was significantly ($P < 0.05$) lower than that of all the other genotypes under study, which did not differ in their leaf ash contents (Figure 6).

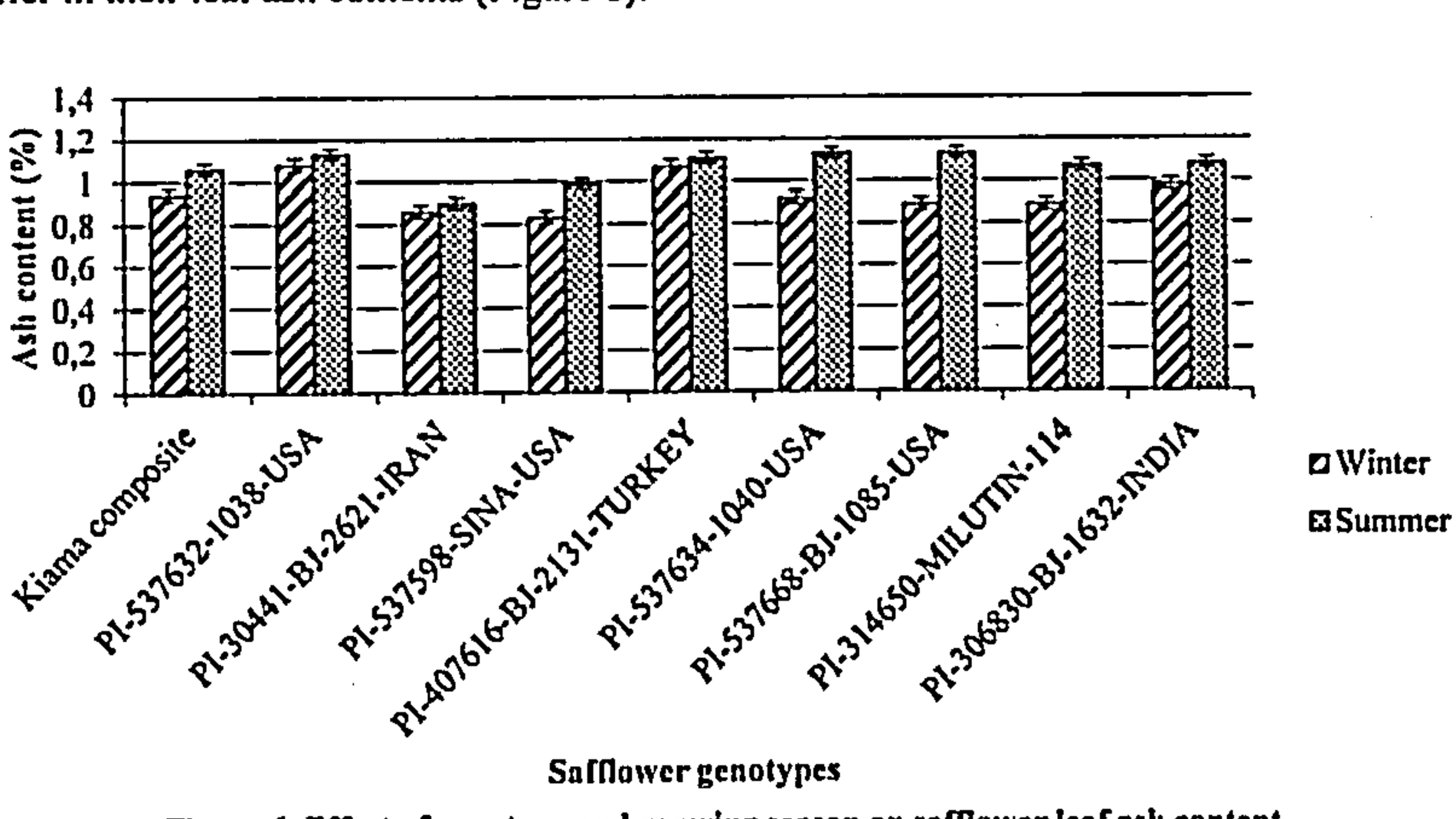

Figure 6. Effect of genotype and growing season on safflower leaf ash content

4.2 Whole seed components

4.2.1 Dry matter

There were significant ($P < 0.05$) differences in whole seed dry matter (DM) depending on the safflower genotype and growing season (Figure 7). The variation in whole seed dry matter ranged between 94.7– 96.1% and 91.9–94.5% in winter and summer grown plants, respectively (Figure 7). With exception of the safflower genotype PI 537598-Sina-USA, all the other genotypes had significantly ($P < 0.05$) higher whole seed DM in winter than summer (Figure 7). Also within season, there was less variation in whole seed DM between genotypes (Figure 7). In winter grown plants, the genotype PI 537632-1038-USA had a whole seed DM of 96.1% which was significantly ($P < 0.05$) higher than the whole seed DM of the genotype PI 537598-Sina-USA (94.5%), but there were no significant ($P > 0.05$) differences in whole seed DM within other genotypes grown in winter (Figure 7). In summer grown plants the genotype PI 537634-1040-USA (94.5%) and PI-537598-Sina-USA (94.5%) maintained their whole seed DM while other cultivars DM decreased (Figure 7). In summer, the genotype PI 407616-BJ-2131-Turkey had a whole seed DM of 91.9% which was significantly ($P < 0.05$) lower than the genotypes PI 537598-Sina-USA and PI 537634-1040-USA which all had a whole seed DM of 94.5% (Figure 7).

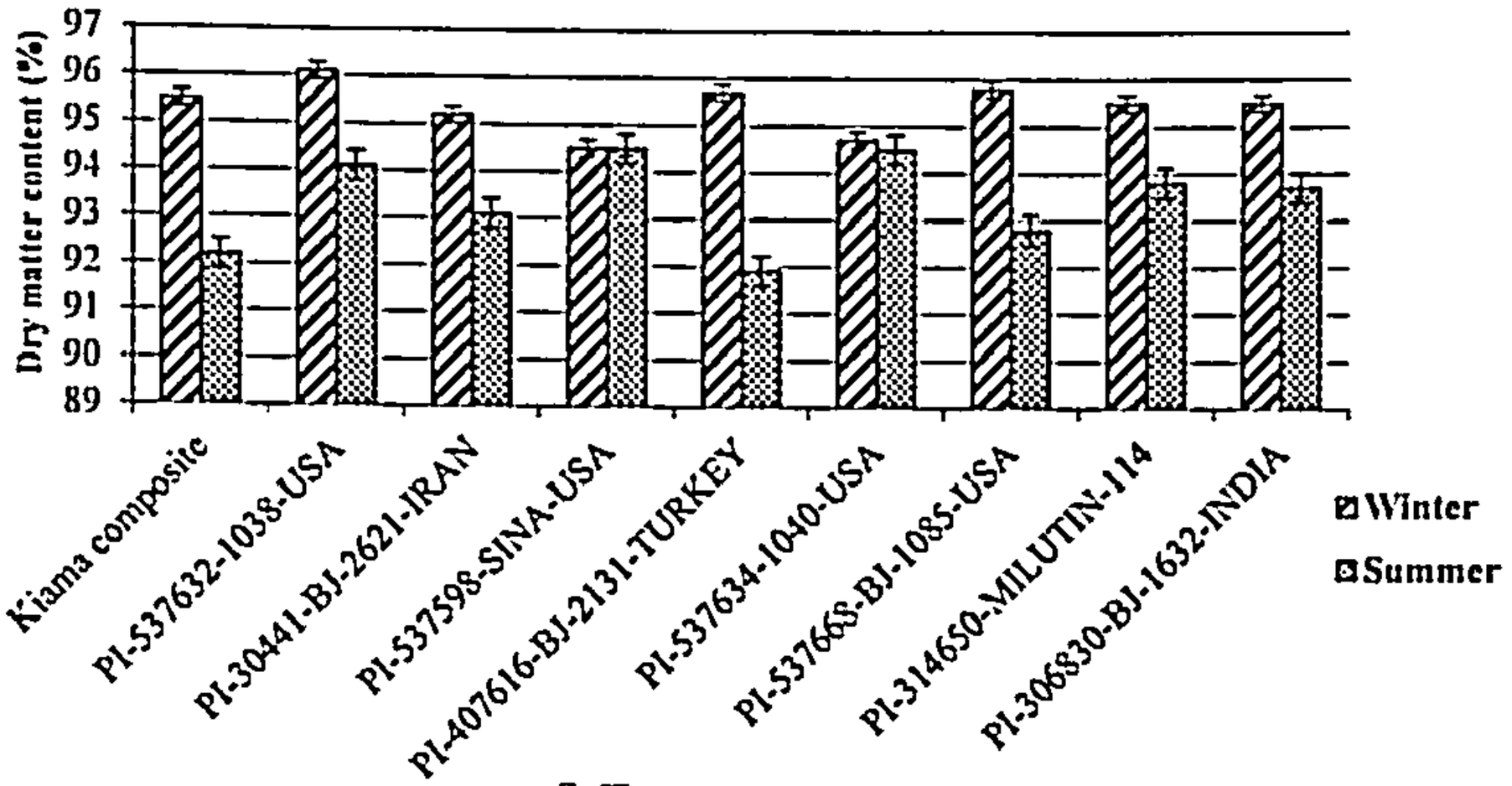

Figure 7. Effect genotype and growing season on safflower whole seed dry matter

4.2.2 Crude protein content

Safflower genotype and growing season significantly ($P < 0.05$) influenced whole seed CP content (Figure 8). The whole seed CP content ranged between 16.3–18.5% and 16.9–19.1% in winter and summer grown plants, respectively (Figure 8). On average the whole seed CP content was only slightly higher in safflower grown in summer (17.6%) than winter (17.4%) (Figure 8). In winter, the genotype PI 407616-BJ-2131-Turkey had the highest whole seed CP content (18.5%) and significantly ($P < 0.05$) higher than that of the genotype PI 314650-Milutin-114-Kazakistan (16.3%), however, the other genotypes did not significantly ($P > 0.05$) differ in their whole seed CP contents (Figure 8). While in summer, the genotype PI 30441-BJ-2521-Iran had a whole seed CP content of 19.1% which was not statistically ($P > 0.05$) different from the whole seed CP contents of the genotypes PI 537632-1038-USA, PI 407616-BJ-2131-Turkey and PI-306830-BJ-1632-India, but was significantly ($P < 0.05$) higher from the other genotypes (Figure 8).

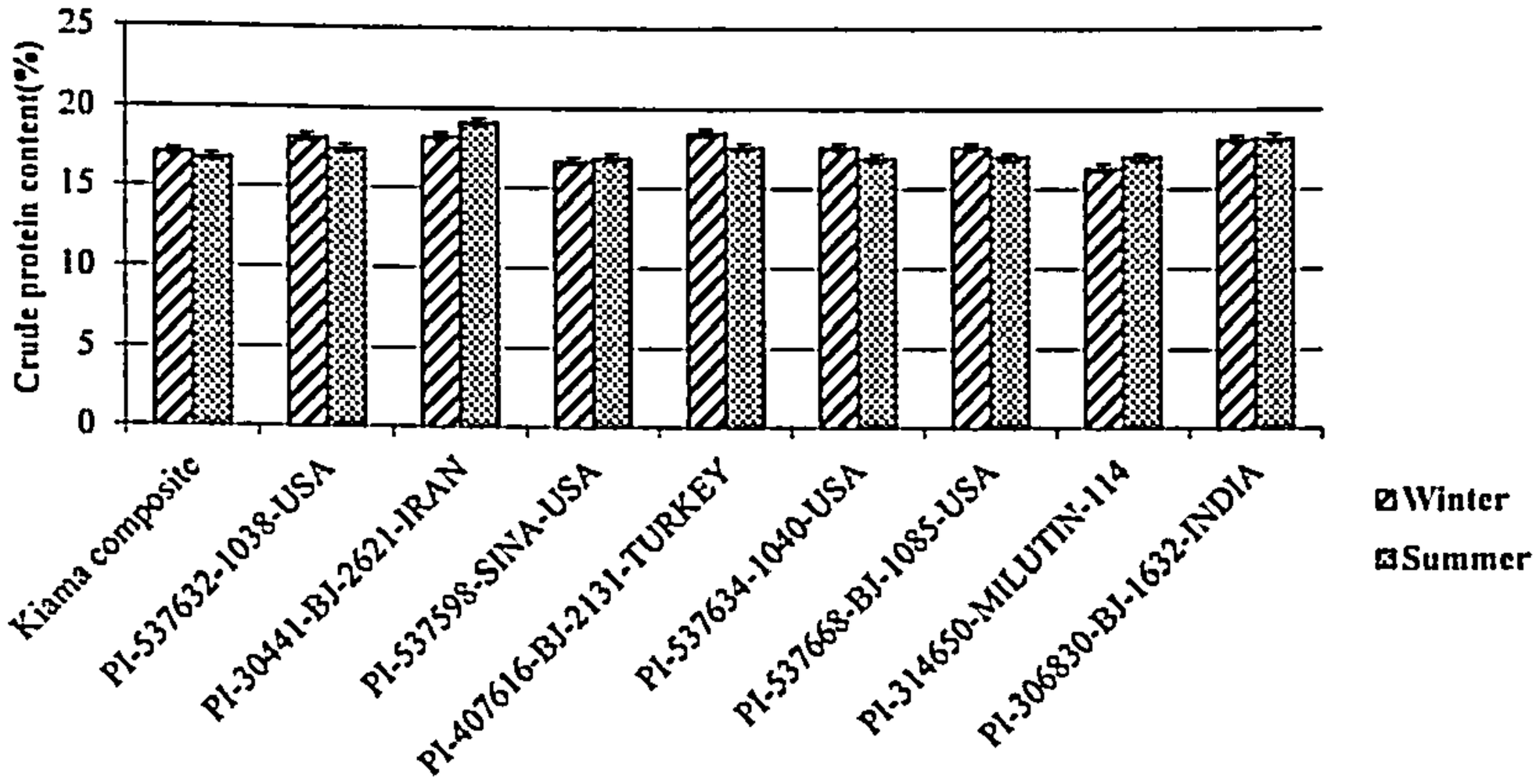

Figure 8. Effect of genotype and growing season on safflower whole seed crude protein

2.3 Neutral detergent fiber

ıe results of the study further showed that genotype and growing season significantly (P < 05) influenced whole seed NDF both in winter and summer grown safflower (Figure 9). The fferences in the whole seed NDF ranged between 46.0–50.3% and 42.6–46.7% in winter and mmer, respectively (Figure 9). In general, whole seed NDF was slightly (P < 0.01) higher in inter season in most safflower genotypes, with an exception of PI 537634-1040-USA which creased by 1.4% in summer (Figure 9). Within the season, PI 537668-BJ-1085-USA (50.3%) id the highest whole seed NDF content in winter and was not significantly (P > 0.01) higher an all other safflower genotypes within the season, excluding PI 30441-BJ-2621-Iran (46.0%) id PI 537634-1040-USA (43.3%) (Figure 9). While in summer, PI 306830-BJ-1632-India and 537668-BJ-1085-USA had an equal whole seed NDF (46.7%) contents (Figure 9). These two notypes were significantly (P < 0.0001) different from PI 407616-BJ-2131-Turkey, PI 537634-)40-USA, PI 537632-1038-USA and PI 30441-BJ-2621-Iran, but not significantly (P > 0.0001)

different from PI 537598-Sina-USA, PI 314650-Milutin-114-Kazakistan and Kiama composite with respect to whole seed NDF (Figure 9).

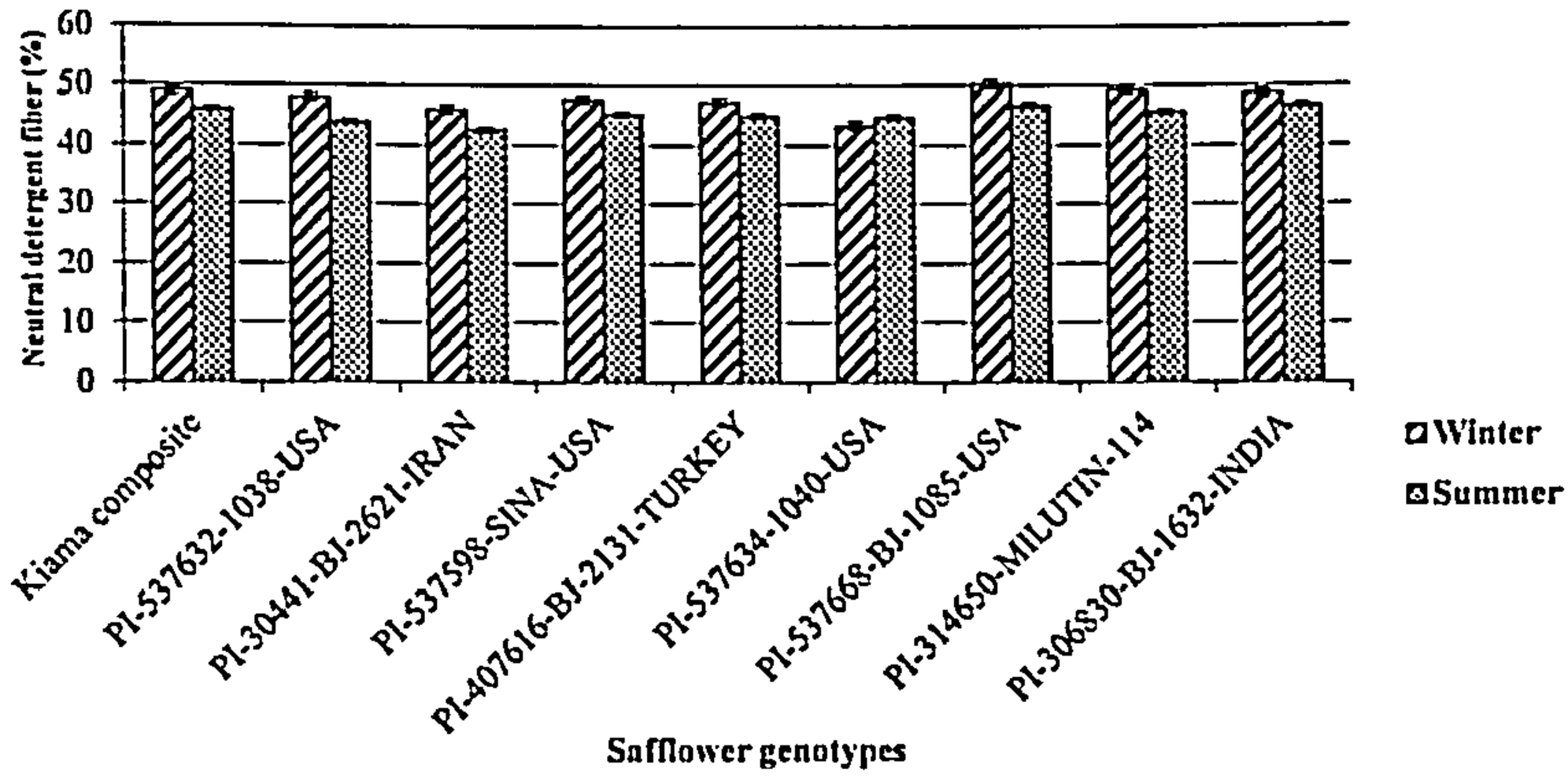

Figure 9. Effect of genotype and growing season on safflower whole seed NDF

4.2.4 Acid detergent fiber

Genotype and growing season significantly ($P < 0.05$) influenced the safflower whole seed ADF (Figure 10). Variation in whole seed ADF ranged between 42.8–48.0% and 39.7–44.6% in winter and summer, respectively (Figure 10). Furthermore, whole seed ADF was significantly ($P < 0.05$) higher in winter than summer growing season (Figure 10). In winter, Kiama composite (48.0%) had the highest whole seed ADF and was not statistically ($P > 0.05$) different from all other varieties apart from PI-306830-BJ-1632-India and PI 314650-Milutin-114-Kazakistan with 43.3 and 42.8% whole seed ADF, respectively (Figure 10). Similarly, in summer, all other safflower genotypes were not statistically ($P > 0.05$) different from each other, but with the exception of PI 537668-BJ-1085-USA, PI 30441-BJ-2621-Iran and PI 407616-BJ-2131-Turkey which were significantly ($P < 0.05$) different from each other (Figure 10).

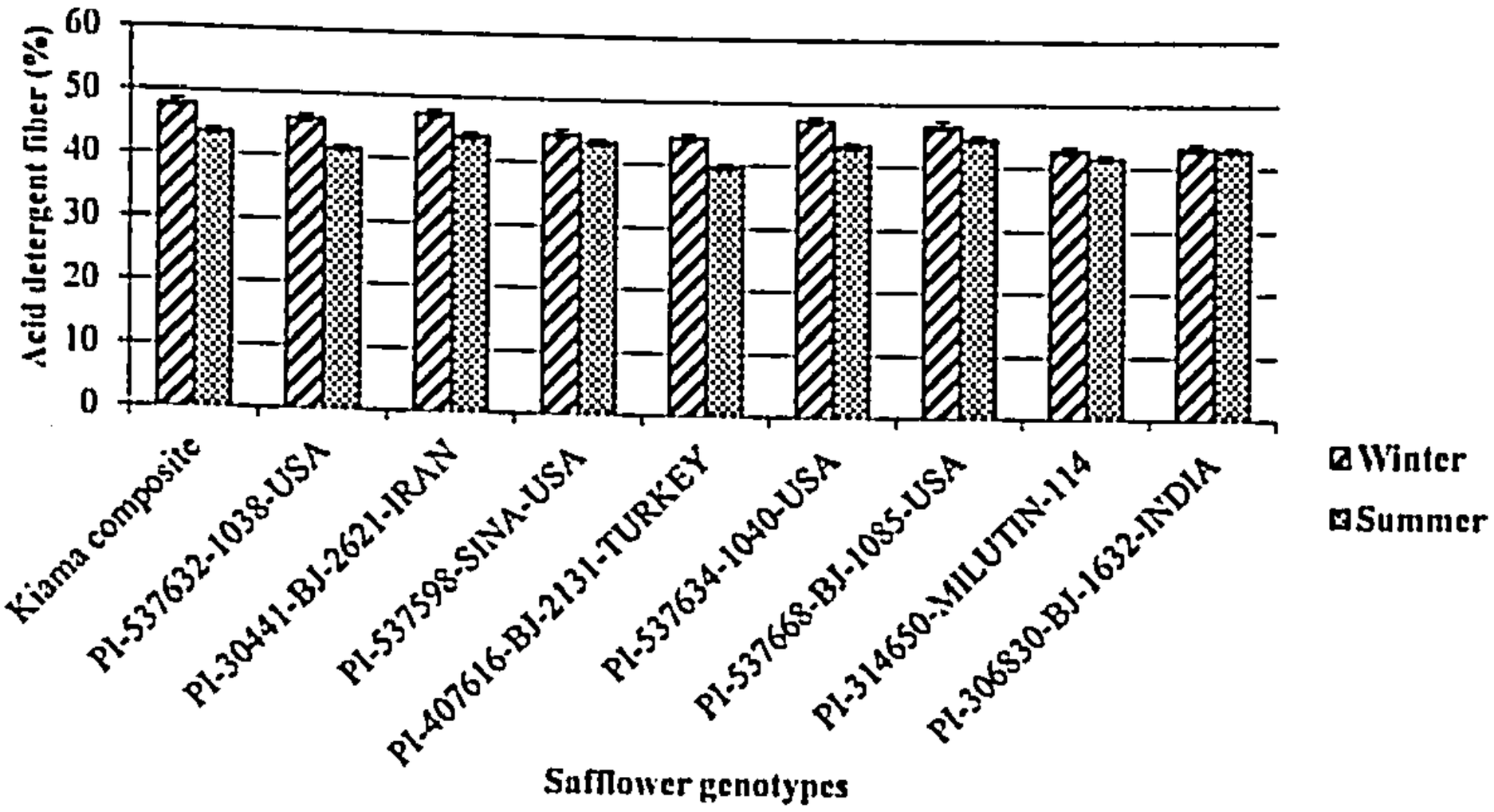

Figure 10. Effect of genotype and growing season on safflower whole seed ADF

4.2.5 Acid detergent lignin

On the other hand, the whole seed ADL significantly ($P < 0.001$) differed depending on the genotype and growing season (Figure 11). The seed ADL ranged between 14.0–20.7% and 13.5–16.0% in winter and summer grown plants, respectively (Figure 11). In winter, the genotype PI 537668-BJ-1085-USA (20.7%) had significantly ($P < 0.001$) higher whole seed ADL than the genotypes PI 407616-BJ-2131-Turkey, PI 314650-Milutin-114-Kazakistan and PI 306830-BJ-1632-India (Figure 11), but not statistically ($P > 0.05$) different from the genotypes PI 537634-1040-USA, PI 537598-Sina-USA, Kiama composite and PI 30441-BJ-2621-Iran (Figure 11). In winter grown safflower, the genotype PI 537598-Sina-USA also had significantly ($P < 0.05$) higher whole seed ADL than the genotypes PI 306830-BJ-1632-India and PI 537632-1038-USA (Figure 11). While in summer grown safflower, there were no significant ($P > 0.05$) differences between safflower genotypes with respect to whole seed ADL contents, with exception of the

genotype PI 30441-BJ-2621-Iran which had significantly ($P < 0.05$) higher whole seed ADL content than the genotype PI 407616-BJ-2131-Turkey (Figure 11).

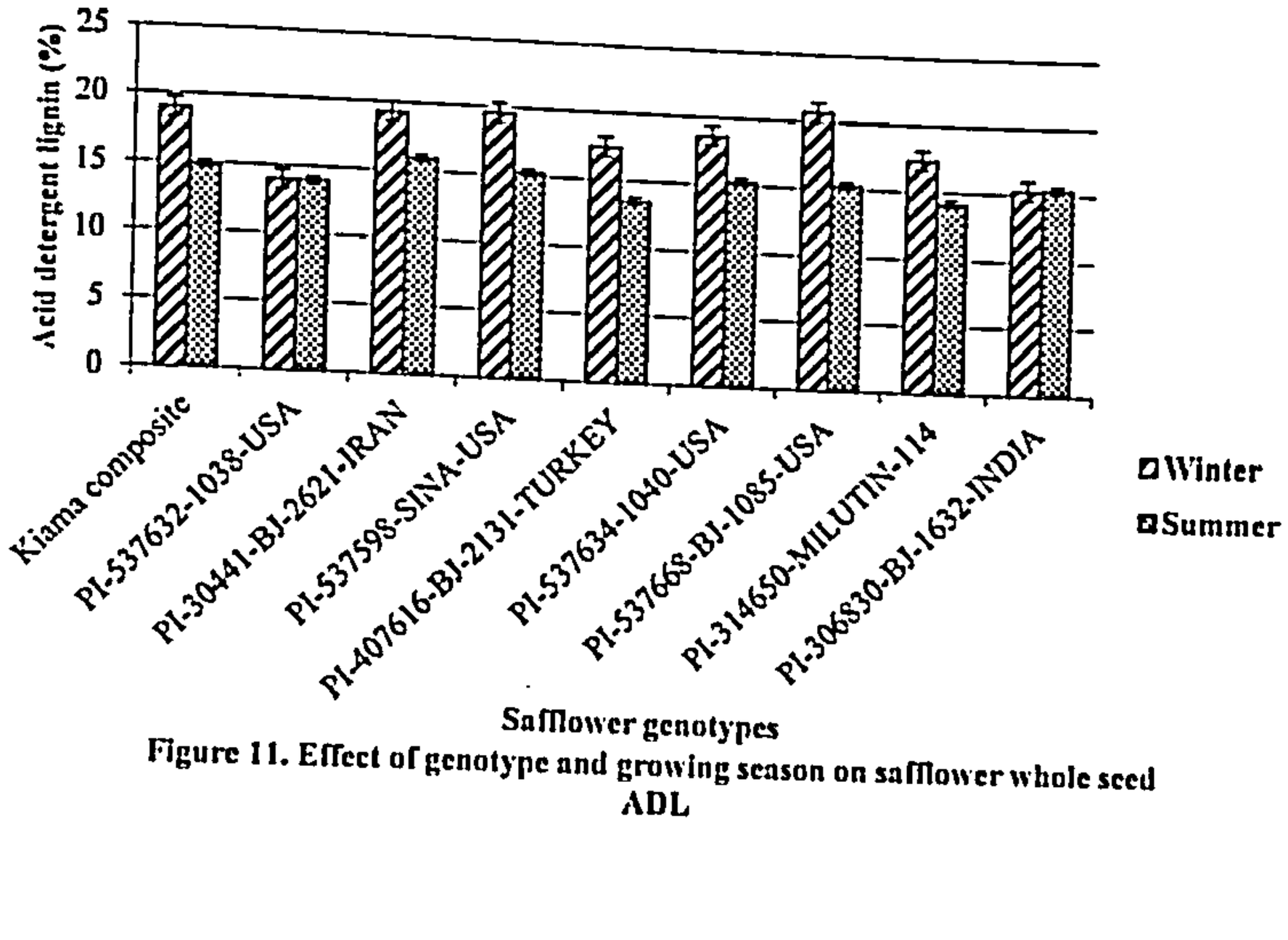

Figure 11. Effect of genotype and growing season on safflower whole seed ADL

4.2.6 Ash content

The whole seed ash content significantly ($P < 0.05$) varied depending on the genotype and growing season (Figure 12). Whole seed ash content was varied between 0.95–1.41% and 1.14–1.24% in winter and summer grown safflower, respectively (Figure 12). Contrary to most seasonal results, safflower genotypes had significantly ($P < 0.05$) higher ash content in summer than in winter in most genotypes (Figure 12). In winter grown safflower, the whole seed ash content of the genotype PI 537634-1040-USA was not significantly ($P > 0.05$) different from that of the genotypes PI 30441-BJ-2621-Iran, PI 537632-1038-USA, PI 407616-BJ-2131-Turkey and PI 537598-Sina-USA, but was significantly ($P < 0.05$) higher than the whole seed ash contents of the genotypes PI 306830-BJ-1632-India, Kiama composite, PI 537668-BJ-1085-USA and PI 314650-Milutin-114-Kazakistan (Figure 12). While in summer, the genotypes Kiama composite

and PI 537634-1040-USA had the highest ash value than other genotypes, but were not statistically ($P > 0.05$) different (Figure 12).

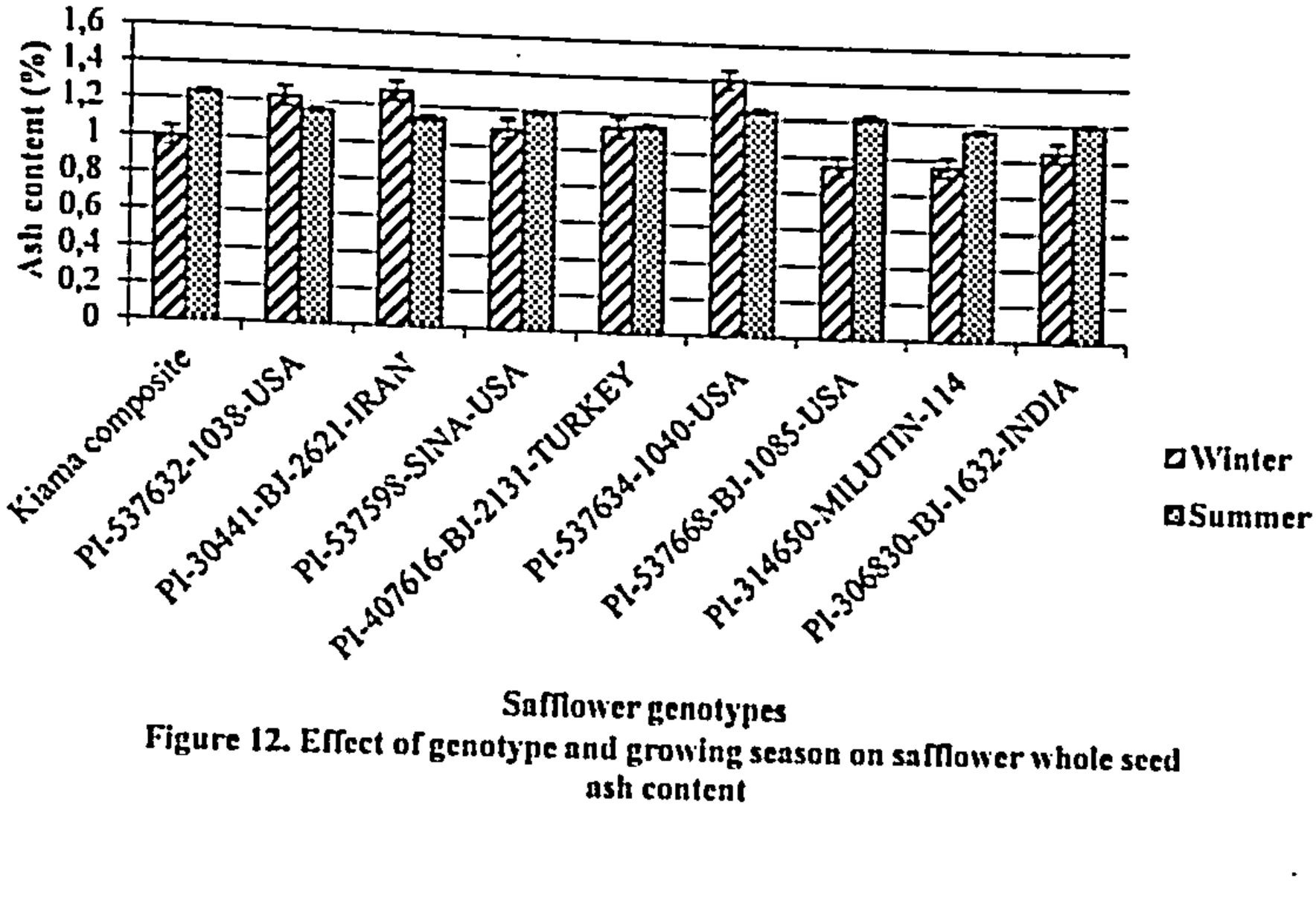

Figure 12. Effect of genotype and growing season on safflower whole seed ash content

4.3 Seed oil content

The results of the study showed that safflower genotypes significantly ($P < 0.0001$) differed in their seed oil contents (Figure 13). The oil seed content of the genotypes varied between 26.1 and 42.2% (Figure 13). The genotype PI 537598-Sina-USA significantly ($P < 0.0001$) produced higher seed oil content (42.2%) than all other safflower genotypes with exception of PI 537632-1038-USA which had a seed oil content of 41% (Figure 13). Furthermore, the genotype PI 314650-Milutin-114-Kazakistan had significantly ($P < 0.0001$) higher seed oil content (36.5%) than the genotypes PI 407616-BJ-2131-Turkey and PI 30441-BJ-2621-Iran with 26.4 and 26.1% seed oil contents, respectively. The genotypes PI 30441-BJ-2621-Iran and PI 407616-BJ-2131-Turkey had significantly ($P < 0.0001$) lower seed oil content of 26.1% and 26.4% respectively than all the other genotypes under study (Figure 13).

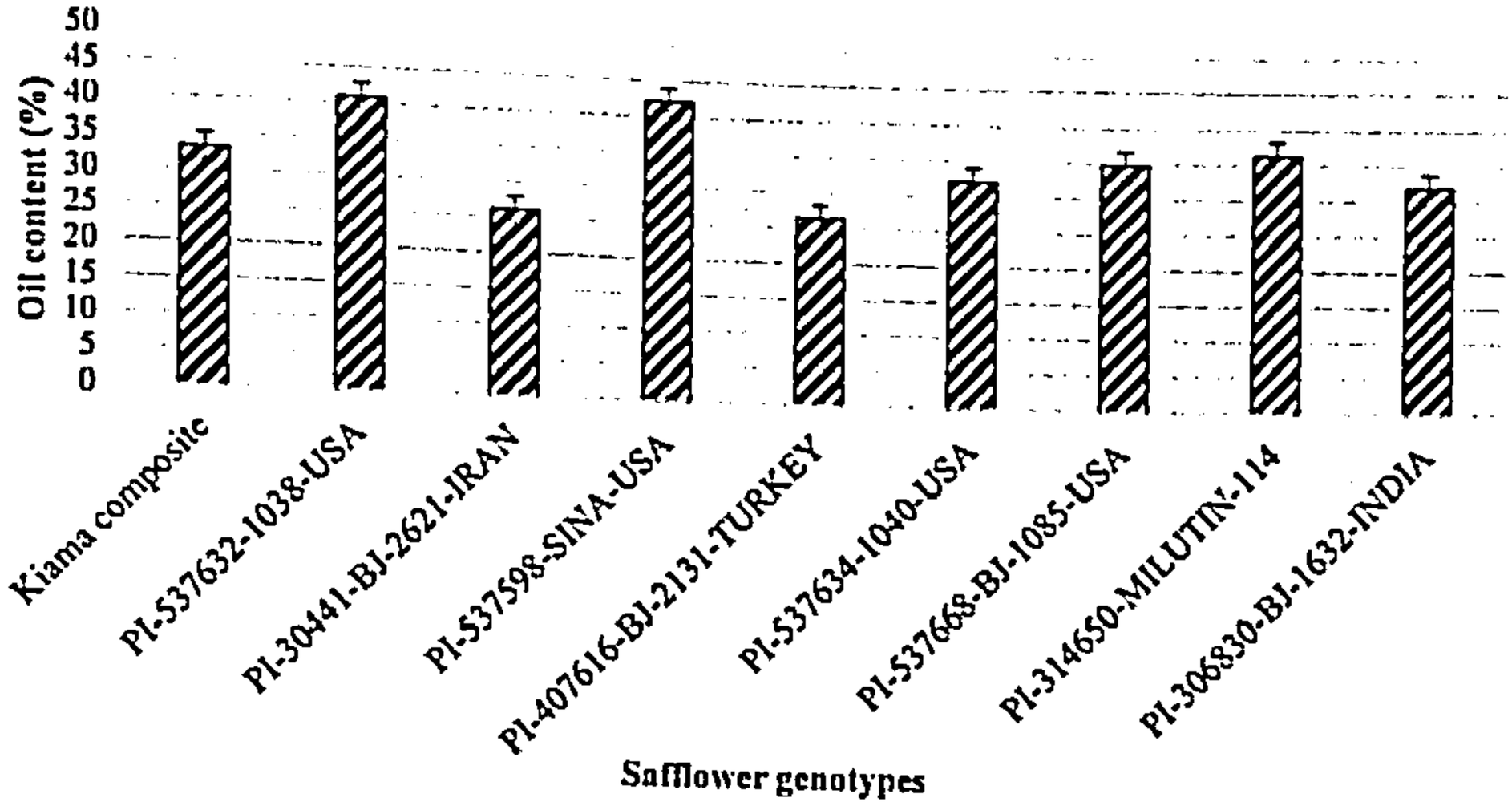

Figure 13. Effect of safflower genotypes on seed oil content (winter)

4.4 Safflower cake components after oil extraction

4.4.1 Crude protein content

There was also a significant ($P < 0.05$) effect of genotype on CP of safflower cake after oil extraction (Figure 14). The CP content in the cake ranged between 19.3 and 22.5% depending on genotype (Figure 14). The genotype PI 537598-Sina-USA (22.5%) had significantly ($P < 0.05$) higher cake CP than all the other genotypes under study (Figure 14). While the genotype PI 306830-BJ-1632-India had significantly ($P < 0.05$) lower cake CP than all other genotypes under study (Figure 14). The other genotypes did not differ significantly ($P > 0.05$) on their cake CP contents, with exception of the genotypes PI 537598-Sina-USA and PI 306830-BJ-1632-India as described above (Figure 14).

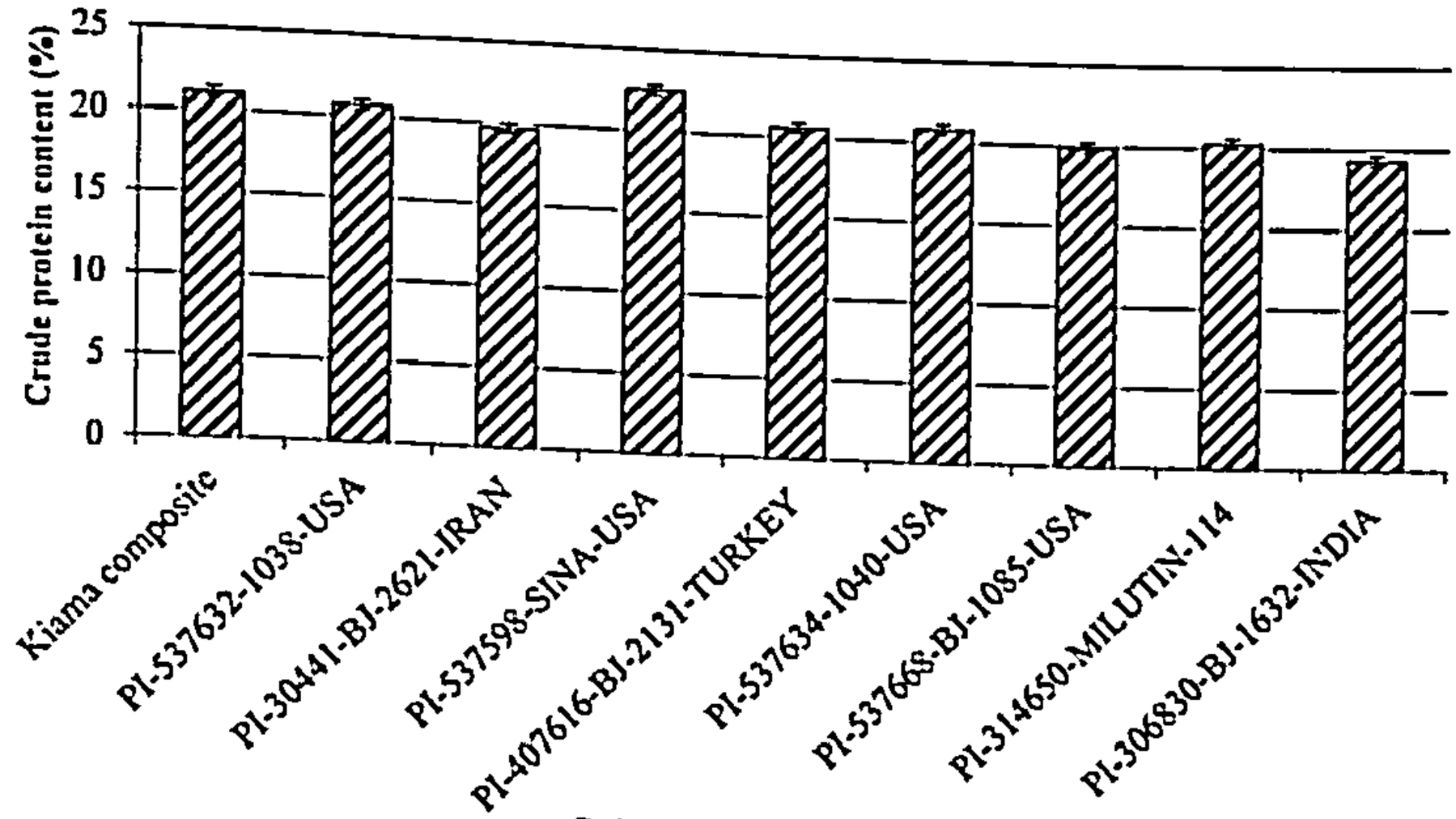

Figure 14. Effect of safflower genotypes on seed cake crude protein (winter)

4.4.2 Neutral detergent fiber

The cake NDF content did not significantly ($P > 0.05$) differ among safflower genotypes (Figure 15). However, the cake NDF among genotypes under study ranged between 54.6 and 61.2% (Figure 15).

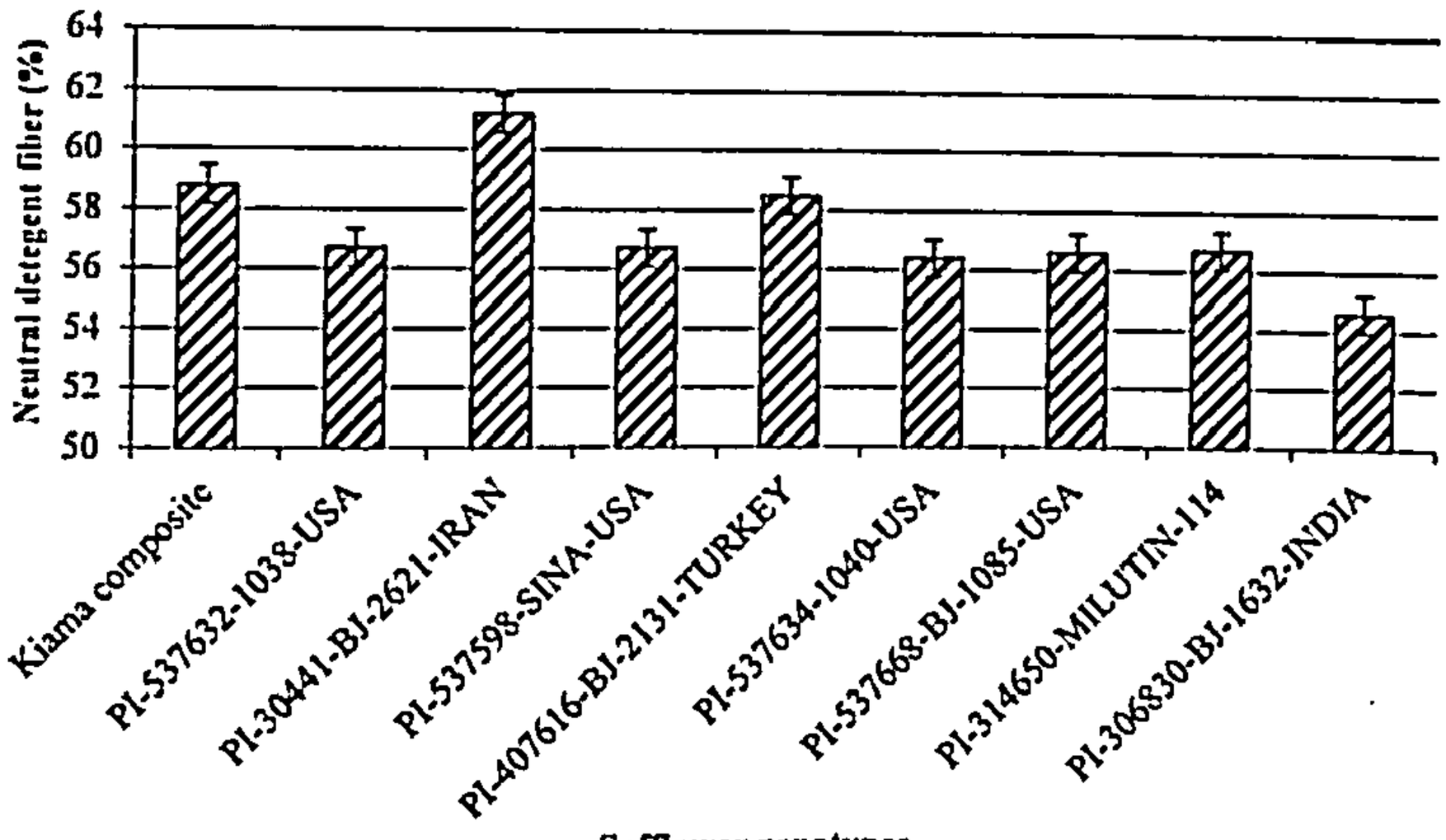

Figure 15. Effect of safflower genotypes on cake NDF (winter)

4.4.3 Acid detergent fiber

The results of the study showed that ADF of safflower cake significantly ($P < 0.05$) varied among genotypes (Figure 16). The cake ADF varied between 45.0 and 50.7% depending on the genotype (Figure 16). The genotypes PI 30441-BJ-2621-Iran and PI 537632-1038-USA had significantly ($P < 0.05$) higher cake ADF than the genotypes PI 537634-1040-USA (Figure 16). The cake ADF of the genotypes PI 30441-BJ-2621-Iran and PI 537632-1038-USA did not significantly ($P > 0.05$) differ from that of the genotypes PI 306830-BJ-1632-India, Kiama composite, PI 407616-BJ-2131-Turkey, PI 314650-Milutin-114-Kazakistan and PI 537598-Sina-USA (Figure 16).

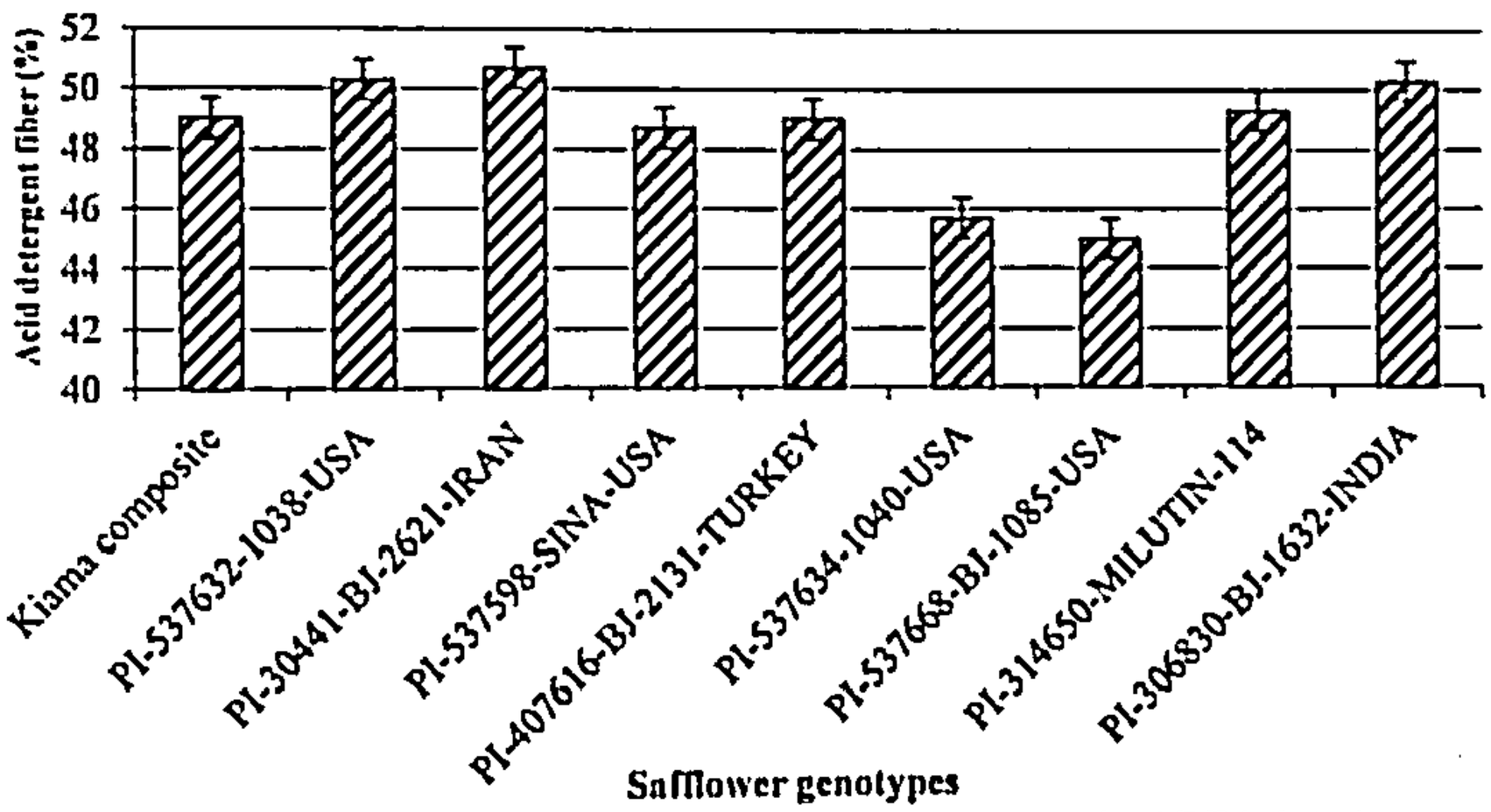

Figure 16. Effect of safflower genotypes on cake ADF (winter)

4.4.4 Acid detergent lignin

No significant ($P < 0.05$) difference was observed in the seed cake ADL content on the safflower genotypes under study (Figure 17). The ADL content ranged from 18.0 and 20.8% (Figure 17). However, Kiama composite had the highest cake ADL of 20.8% while the genotype PI 306830-BJ-1632-India had the lowest seed cake ADL of 18.0% (Figure 17).

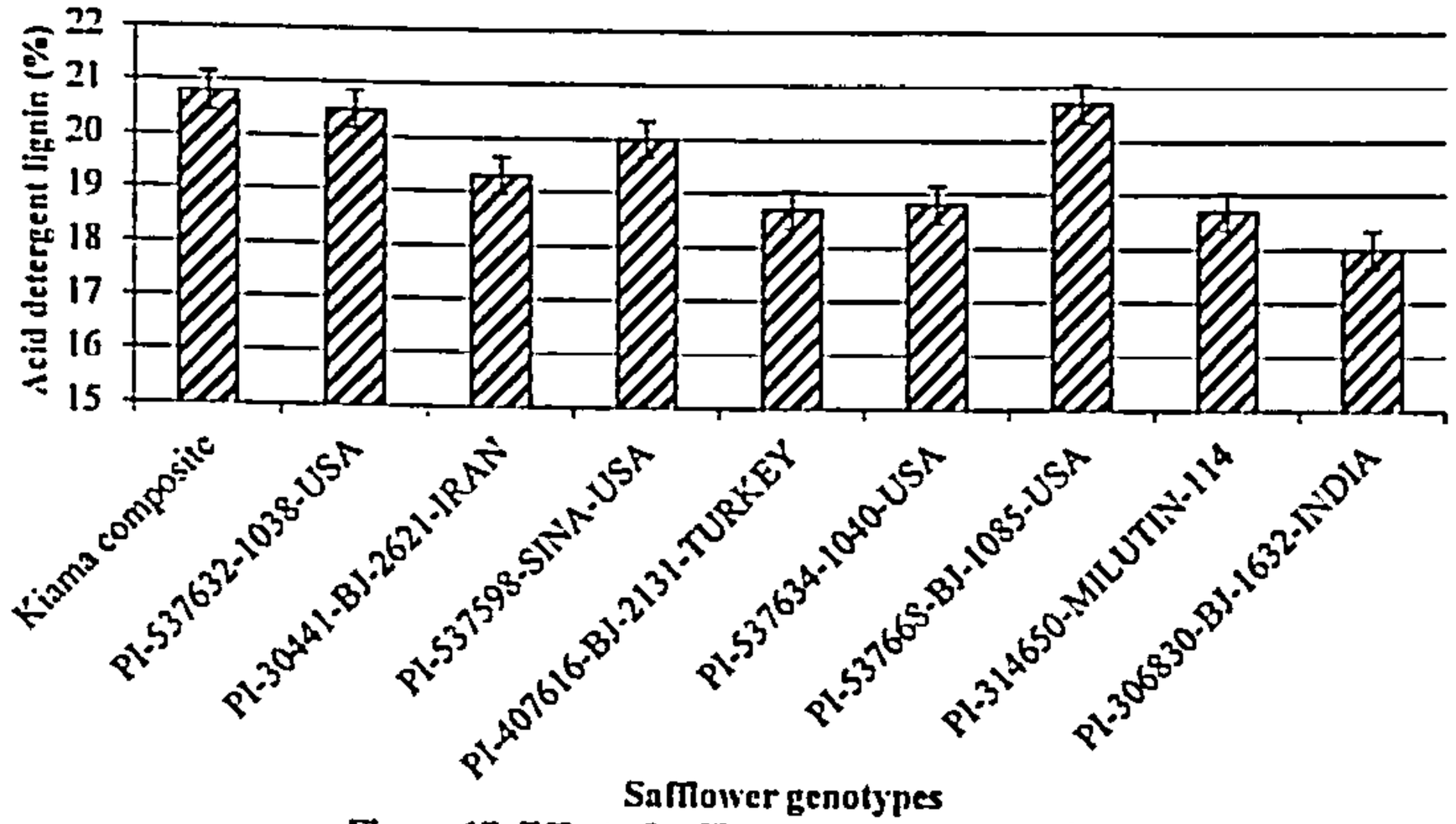

Figure 17. Effect of safflower genotypes on cake ADL (winter)

4.3.5 Ash content

The cake ash content of the different safflower genotypes did not significantly ($P > 0.05$) differ (Figure 18). However, the seed cake ash content of the genotypes varied between 1.10 and 1.60% in safflower cakes (Figure 18).

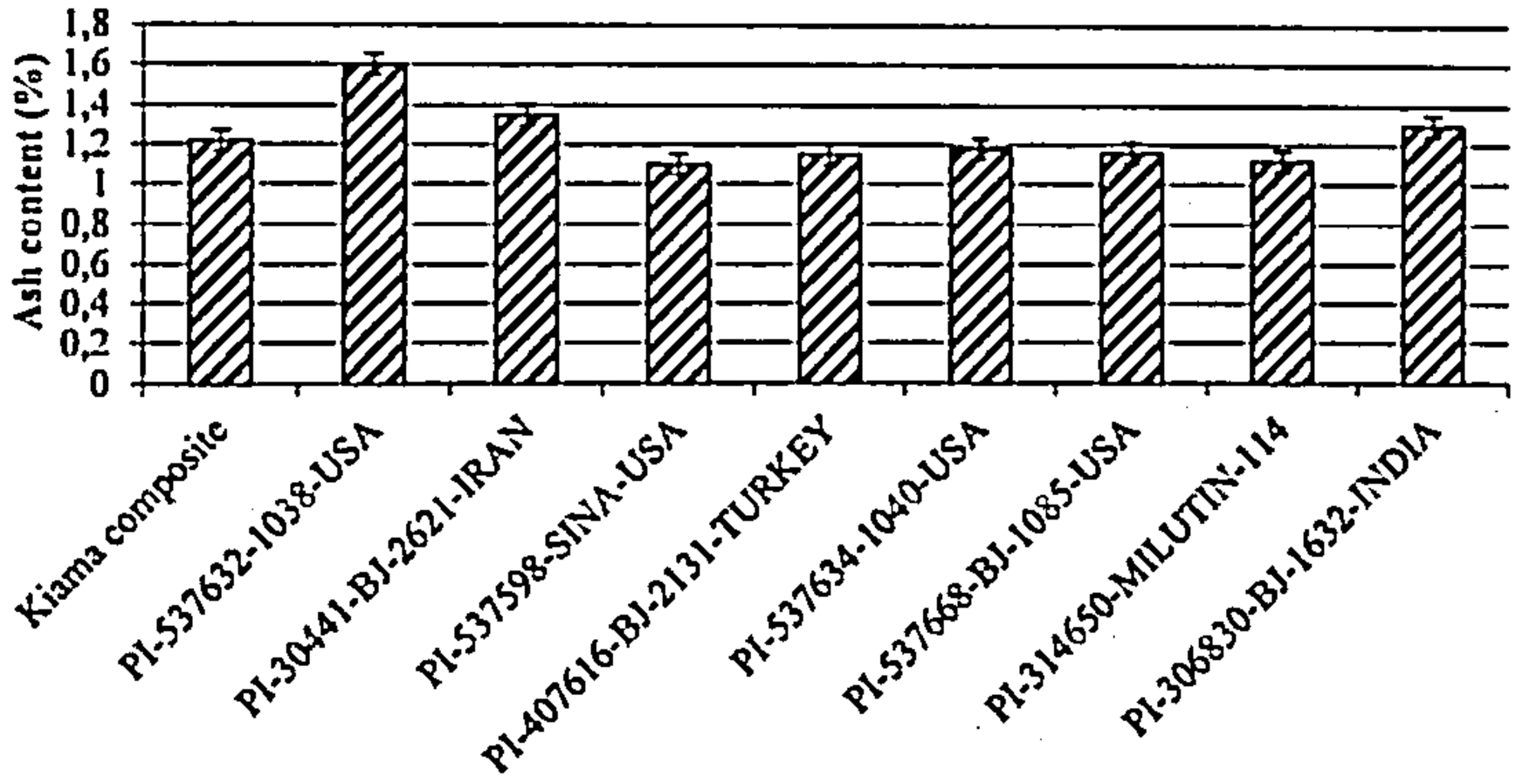

Figure 18. Effect of safflower genotypes on cake ash content (winter)

linerals

Phosphorus content

.1 Leaf

safflower leaf phosphorus (P) content significantly ($P < 0.05$) varied with genotype (Table he leaf P content ranged between 3.31 and 4.95 mg/g (Table 1). The genotype PI 407616-131-Turkey with the highest leaf P content of 4.95 mg/g was significantly ($P < 0.05$) higher that of the genotype PI 306830-BJ-1632-India, but was not different from the leaf P nts of the other genotypes under study (Table 1). However, the leaf P content of the ype PI 306830-BJ-1632-India did not significantly ($P > 0.05$) differ from that of the ypes PI 314650-Milutin-114-Kazakistan, PI 537632-1038-USA, PI 537598-Sina-USA and 7668-BJ-1085-USA (Table1).

2 Whole seed

content of safflower whole seed significantly ($P < 0.01$) varied with genotype (Table 1). content in whole seed varied between 5.47 and 7.87 mg/g depending on safflower ypes (Table 1). The genotype PI 306830-BJ-1632-India had the highest P content (7.87 in safflower whole seed and it was significantly ($P < 0.01$) higher than whole seed P nts of the genotypes Kiama composite, PI 537632-1038-USA, PI 30441-BJ-2621-Iran and 7668-BJ-1085-USA (Table 1). However, the whole seed P content of the genotype PI 0-BJ-1632-India did not significantly ($P > 0.05$) differ with that of the genotypes PI 0-Milutin-Kazakistan, PI 537634-1040-USA, PI 407616-BJ-2131-Turkey and PI 537598-JSA (Table 1). Also the whole seed P content of the genotypes Kiama composite, PI -BJ-2621-Iran, PI 537668-BJ-1085-USA, PI 407616-BJ-2131-Turkey, PI 537632-1038-

USA, PI 537598-Sina-USA and PI 537634-1040-USA did not significantly (P > 0.05) differ (Table 1).

4.5.1.3 Cake

The P cake content of safflower genotypes significantly (P < 0.01) differed (Table 1). The P content of the different safflower genotypes cake ranged between 6.98 and 7.90 mg/g (Table 1). The genotype PI 537668-BJ-1085-USA had significantly (P < 0.01) higher cake P content (7.90 mg/g) than PI 407616-BJ-2131-Turkey, PI 314650-Milutin-114-Kazakistan, PI 537632-1038-USA and PI-306830-BJ-1632-India (Table 1). Also the P contents of the genotypes PI 537668-BJ-1085-USA, Kiama composite, PI 537598-Sina-USA, PI 537634-1040-USA and PI 30441-BJ-2621-Iran did not significantly (P > 0.05) differ (Table 1). The safflower genotype PI 537632-1038-USA had the lowest cake P content (6.98 mg/g), but was not significantly (P > 0.05) different that of the genotypes Kiama composite, PI 407616-BJ-2131-Turkey, PI 314650-Milutin-114-Kazakistan and PI-306830-BJ-1632-India (Table 1).

Table 1. Phosphorus content of safflower leaf, whole seed and cake

Genotype	Phosphorus content (mg/g)		
	Leaf	Whole seed	Cake
Kiama composite	4.66a	6.20bc	7.46abc
PI-537632-1038-USA	4.32ab	6.08bc	6.98c
PI-30441-BJ- 2621-IRAN	4.67a	5.65c	7.68ab
PI-537598-SINA-USA	3.97ab	6.60abc	7.85a
PI-407616-BJ-2131-TURKEY	4.95a	6.61abc	7.17c
PI-537634-1040-USA	4.71a	6.77abc	7.75a
PI-537668-BJ-1085-USA	4.00ab	5.47c	7.90a
PI-314650-MILUTIN-114-KAZAKISTAN	4.08ab	7.08ab	7.08c
PI-306830-BJ-1632-INDIA	3.31b	7.87a	7.19bc
Significance	*	**	**
LSD	1.30	1.32	0.49

*, ** Significant at P = 0.05, 0.01, respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.2 Potassium content

4.5.2.1 Leaf

The results of the study showed that genotypes significantly ($P < 0.05$) differed in their leaf potassium (K) content (Table 2). The leaf K content ranged between 56.13 and 66.54 mg/g (Table 2). The genotype PI 537598-Sina-USA had the highest leaf K content of 66.54 mg/g and was significantly ($P < 0.05$) than the leaf K content of the genotype Kiama composite (Table 2). However, the leaf K content of the genotype PI 537598-Sina-USA was not significantly ($P > 0.05$) different from that of other genotypes, with exception of Kiama Composite (Table 2).

4.5.2.2 Whole seed

There were also significant ($P < 0.0001$) differences in the whole seed K content of the genotypes (Table 2). The K content of the genotypes varied between 8.44 and 12.26 mg/g (Table 2). The genotype PI 306830-BJ-1632-India had the highest whole seed K content of 12.26 mg/g and it was significantly ($P < 0.0001$) higher than that of the genotypes PI 314650-Milutin-114-Kazakistan, Kiama composite, PI 537668-BJ-1085-USA, PI 407616-BJ-2131-Turkey and PI 30441-BJ-2621-Iran (Table 2). However, the whole K seed content of the genotype PI 306830-BJ-1632-India did not significantly ($P > 0.05$) differ with that of the genotypes PI 537634-1040-USA, PI 537598-Sina-USA and PI 537632-1038-USA (Table 2). The genotype PI 30441-BJ-2621-Iran had the lowest whole seed K content of 8.44 mg/g but was not significantly ($P > 0.05$) different from the whole seed K content of the genotype PI 407616-BJ-2131-Turkey (Table 2).

4.5.2.3 Cake

Safflower genotypes significantly ($P < 0.05$) influenced the K content of the cake after oil extraction (Table 2). The cake K content ranged between 10.68 and 12.91 mg/g (Table 2). The

genotype PI 537598-Sina-USA had the highest cake K content of 12.91 mg/g K, but was not statistically ($P > 0.05$) higher than that of other genotypes, with exception of the genotype PI-314650-Milutin-114-Kazakistan which had a significantly ($P < 0.05$) low cake K content of 10.69 mg/g (Table 2).

Table 2. Potassium content of safflower leaf, whole seed and cake

Genotypes	Potassium content (mg/g)		
	Leaf	Whole seed	Cake
Kiama composite	56.13b	10.25bc	11.49ab
PI-537632-1038-USA	63.21ab	10.92ab	12.13ab
PI-30441-BJ- 2621-IRAN	63.25ab	8.44d	11.89ab
PI-537598-SINA-USA	66.54a	11.13ab	12.91a
PI-407616-BJ-2131-TURKEY	64.34ab	9.34dc	10.69b
PI-537634-1040-USA	65.04ab	12.04a	12.12ab
PI-537668-BJ-1085-USA	61.52ab	9.91bc	11.40ab
PI-314650-MILUTIN-114-KAZAKISTAN	63.82ab	10.27bc	10.94b
PI-306830-BJ-1632-INDIA	63.07ab	12.26a	11.22ab
Significance	*	****	*
LSD	8.91	1.36	1.76

*, **** Significant at P = 0.05, 0.0001 respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.3 Calcium content

4.5.3.1 Leaf

The leaf calcium (Ca) content of safflower significantly ($P < 0.0001$) differed with genotype (Table 3). The leaf Ca content ranged from 10.61 to 16.51 mg/g depending on safflower genotype (Table 3).The genotypes PI 306830-BJ-1632-India and PI 537668-BJ-1085-USA had significantly ($P < 0.0001$) higher leaf Ca content than all other genotypes under study, but did not significantly ($P > 0.05$) differ in their leaf Ca content (Table 3). The genotype PI 537634-1040-USA had the lowest leaf Ca content of 10.61 mg/g, but was not significantly ($P > 0.05$)

different from the leaf Ca content of the genotypes PI 537632-1038-USA, Kiama composite, PI 537598-Sina-USA, PI 314650-Milutin-114-Kazakistan and PI 30441-BJ-2621-Iran (Table 3).

4.5.3.2 Whole seed

Safflower genotype significantly ($P < 0.05$) influenced the whole seed Ca content (Table 3). The whole seed Ca content ranged between 9.45 and 11.33 mg/g (Table 3). The genotype PI 306830-BJ-1632-India had a whole seed Ca content of 11.33 mg/g which was significantly ($P < 0.05$) higher than that of the genotypes Kiama composite and PI 407616-BJ-2131-Turkey, but was not significantly ($P > 0.05$) different from the whole seed Ca content of other genotypes (Table 3). PI 537668-BJ-1085-USA, PI 537634-1040-USA with the highest value of calcium contents were significantly ($P < 0.05$) higher than Kiama composite and PI 407616-BJ-2131-Turkey only, hence not significantly ($P > 0.05$) higher than PI 537632-1038-USA, PI 314650-Milutin-114-Kazakistan, PI 537598-Sina-USA and PI 30441-BJ-2621-Iran (Table 3). The genotype Kiama composite had the lowest whole seed Ca content of 9.45 mg/g, but was not significantly ($P > 0.05$) different from the whole seed Ca content of the genotypes Kiama composite, PI 537632-1038-USA, PI 314650-Milutin-114-Kazakistan, PI 537598-Sina-USA and PI 30441-BJ-2621-Iran (Table 3).

4.5.3.3 Cake

The cake Ca content did not significantly ($P > 0.05$) differ with genotype (Table 3). The Ca content of safflower cake after oil extraction ranged between 8.78 and 10.61 mg/g (Table 3).

Table 3. Calcium content of safflower leaf, whole seed and cake

Genotype	Calcium content (mg/g)		
	Leaf	Whole seed	Cake
Kiama composite	12.03dc	9.50b	8.78a
PI-537632-1038-USA	12.66bc	10.53ab	9.50a
PI-30441-BJ- 2621-IRAN	11.31dc	9.92ab	10.05a
PI-537598-SINA-USA	11.96dc	9.95ab	9.53a
PI-407616-BJ-2131-TURKEY	13.72b	9.45b	9.74a
PI-537634-1040-USA	10.61d	11.06a	10.61a
PI-537668-BJ-1085-USA	15.32a	11.16a	10.07a
PI-314650-MILUTIN-114-KAZAKISTAN	11.88dc	10.48ab	10.53a
PI-306830-BJ-1632-INDIA	16.51a	11.33a	10.39a
Significance	****	*	NS
LSD	1.59	1.43	1.85

*, ****, NS. Significant at P = 0.05, 0.0001 or non-significant, respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.4 Magnesium content

4.5.4.1 Leaf

The leaf magnesium (Mg) content of safflower genotypes was not significantly ($P > 0.05$) different (Table 4). The leaf Mg content ranged from 3.91 to 4.92 mg/g depending on genotype (Table 4).

4.5.4.2 Whole seed

Safflower genotype significantly ($P < 0.05$) influenced the whole seed Mg content (Table 4). The whole seed Mg content ranged between 4.37 and 5.55 mg/g depending on genotype (Table 4). The genotype PI 306830-BJ-1632-India had a whole seed Mg content of.55 mg/g which was significantly ($P < 0.05$) higher than the whole seed Mg content of the genotypes PI 407616-BJ-2131-Turkey, Kiama composite , PI 30441-BJ-2621-Iran and PI 537668-BJ-1085-USA which had whole seed Mg contents of 4.72, 4.69, 4.54 4.37 mg/g, respectively (Table 4). However,

the whole seed Mg content of the genotype PI 306830-BJ-1632-India was not significantly (P > 0.05) different from that of the genotypes PI 537598-Sina-USA (5.38 mg/g), PI 314650-Milutin-114-Kazakistan (5.28 mg/g), PI 537634-1040-USA (4.80 mg/g) and PI 537632-1038-USA (4.75 mg/g) (Table 4). The genotype PI 537668-BJ-1085-USA had the lowest whole seed Mg content of 4.37 mg/g, but was not significantly (P > 0.05) different from the whole seed Mg contents of the genotypes Kiama composite, PI 537634-1040-USA, 537632-1038-USA, PI 407616-BJ-2131-Turkey and PI 30441-BJ-2621-Iran (Table 4).

4.5.4.3 Cake

Safflower genotypes did not significantly (P > 0.05) differ in their cake Mg contents (Table 4). The cake Mg content ranged between 4.45 and 4.99 mg/g depending on genotype (Table 4). Similarly as in whole seed, the genotype PI 306830-BJ-1632-India had the highest Mg content, but it was not significantly (P > 0.05) different from that of other genotypes (Table 4).

Table 4. Magnesium content of safflower leaf, whole seed and cake

Genotype	Magnesium content (mg/g)		
	Leaf	Whole seed	Cake
Kiama composite	4.14a	4.69bcd	4.51a
PI-537632-1038-USA	3.95a	4.75abdc	4.45a
PI-30441-BJ- 2621-IRAN	4.31a	4.54dc	4.49a
PI-537598-SINA-USA	3.92a	5.38ab	4.56a
PI-407616-BJ-2131-TURKEY	4.92a	4.72bcd	4.74a
PI-537634-1040-USA	4.04a	4.80abcd	4.74a
PI-537668-BJ-1085-USA	4.49a	4.37d	4.46a
PI-314650-MILUTIN-114-KAZAKISTAN	3.91a	5.28abc	4.93a
PI-306830-BJ-1632-INDIA	4.36a	5.55a	4.99a
Significance	NS	*	NS
LSD	1.03	0.81	1.03

*, NS Significant at P = 0.05 or non-significant, respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.5 Sodium content

4.5.5.1 Leaf

In safflower leaves, genotypes significantly ($P < 0.01$) differed in their sodium (Na) contents (Table 5). The leaf Na content ranged between 0.51 and 0.69 mg/g depending on genotype (Table 5). The genotype PI 537632-1038-USA had significantly ($P < 0.01$) higher leaf Na content than the genotypes Kiama composite, PI 537634-1040-USA, PI 407616-BJ-2131-Turkey, PI 306830-BJ-1632-India and PI 314650-Milutin-114-Kazakisatan (Table 5). However, the leaf Na content of the genotype PI 537632-1038-USA was not significantly ($P > 0.05$) different from that of the genotypes PI 537598-Sina-USA, PI 30441-BJ-2621-Iran and PI 537668-BJ-1085-USA (Table 5). Also the leaf Na contents of the genotypes Kiama composite, PI 537634-1040-USA, PI 407616-BJ-2131-Turkey, PI 306830-BJ-1632-India and PI 314650-Milutin-114-Kazakisatan did not statistically differ (Table 5).

4.5.5.2 Whole seed

The Na content of safflower whole seed significantly ($P < 0.05$) differed depending on genotype (Table 5). The whole seed Na content ranged between 3.24-3.57 mg/g, depending genotype (Table 5). The genotype PI 537598-Sina-USA had significantly ($P < 0.05$) higher whole seed Na content than the genotypes PI 407616-BJ-2131-Turkey, PI 537634-1040-USA and PI 30441-BJ-2621-Iran (Table 5). However, the genotype PI 537598-Sina-USA which had the highest whole seed Na content of 3.57 mg/g was not significantly ($P > 0.05$) different from the whole seed Na content of the genotypes Kiama composite, PI 537632-1038-USA, PI 537668-BJ-1085-USA, PI 306830-BJ-1632-India and PI 314650-Milutin-114-Kazakisatan (Table 5). The genotype PI 537634-1040-USA had the lowest whole seed Na content of 3.24 mg/g, but was not significantly

(P > 0.05) from the whole seed Na content of the genotypes Kiama composite, PI 537632-1038-USA, PI 30441-BJ-2621-Iran, PI 537668-BJ-1085-USA, PI 407616-BJ-2131-Turkey and PI 314650-Milutin-114-Kazakisatan (Table 5).

4.5.5.3 Cake

The cake Na content of safflower genotypes significantly (P < 0.05) differed (Table 5). The cake Na content of safflower genotypes ranged between 3.24 and 3.70 mg/g (Table 5). The genotype PI 407616-BJ-2131-Turkey had a cake Na content of 3.70 mg/g which was significantly (P < 0.05) higher than that of all the other genotypes (Table 5). With exception of the cake Na content of the genotype PI 407616-BJ-2131-Turkey, the cake content of the other genotypes did not significantly (P > 0.05) differ from each other (Table 5).

Table 5. Sodium content of safflower leaf, whole seed and cake

	Sodium content (mg/g)		
Genotype	Leaf	Whole seed	Cake
Kiama composite	0.51b	3.42abc	3.30b
PI-537632-1038-USA	0.69a	3.41abc	3.24b
PI-30441-BJ- 2621-IRAN	0.61ab	3.24c	3.29b
PI-537598-SINA-USA	0.67a	3.57a	3.26b
PI-407616-BJ-2131-TURKEY	0.54b	3.31bc	3.70a
PI-537634-1040-USA	0.54b	3.28c	3.29b
PI-537668-BJ-1085-USA	0.59ab	3.45abc	3.27b
PI-314650-MILUTIN-114-KAZAKISTAN	0.53b	3.40abc	3.30b
PI-306830-BJ-1632-INDIA	0.54b	3.54ab	3.26b
Significance	**	*	*
LSD	0.10	0.24	0.31

*, ** Significant at P = 0.05, 0.01. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.6 Zinc content

4.5.6.1 Leaf

The leaf zinc (Zn) content of safflower genotypes did not significantly ($P > 0.05$) differ from each other (Table 6). The leaf Zn content of the genotypes ranged between 70 and 90 µg/g (Table 6).

4.5.6.2 Whole seed

The Zn content of safflower whole seed significantly ($P < 0.05$) varied depending on genotype (Table 6). The whole seed Zn content of safflower seeds ranged between 90 and 120 µg/g (Table 6). The safflower genotypes PI 306830-BJ-1632-India and PI 537598-Sina-USA had a similar whole seed Zn content of 120 µg/g which was significantly ($P < 0.05$) higher than the whole seed Zn content of the genotype PI 30441-BJ-2621-Iran, but was not significantly ($P > 0.05$) different from the whole seed Zn content of the other genotypes (Table 6).

4.5.6.3 Cake

The cake Zn content of safflower genotypes under study did not significantly ($P > 0.05$) differ (Table 6). The Zn content of cake ranged between 90-120 µg/g depending on the genotype (Table 6).

Table 6. Zinc contents of safflower leaf, whole seed and cake

Genotype	Zinc content (µg/g)		
	Leaf	Whole seed	Cake
Kiama composite	90a	110ab	100a
PI-537632-1038-USA	70a	110ab	100a
PI-30441-BJ- 2621-IRAN	90a	90b	90a
PI-537598-SINA-USA	90a	120a	100a
PI-407616-BJ-2131-TURKEY	90a	110ab	120a
PI-537634-1040-USA	70a	100ab	120a
PI-537668-BJ-1085-USA	70a	100ab	110a
PI-314650-MILUTIN-114-KAZAKISTAN	70a	100ab	110a
PI-306830-BJ-1632-INDIA	80a	120a	110a
Significance	NS	*	NS
LSD	30	20	44

*, NS, Significant at P = 0.05 or non-significant, respectively. Means with the same letter(s) within the column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.7 Iron content

4.5.7.1 Leaf

The leaf iron (Fe) content of safflower significantly ($P < 0.01$) differed with genotype (Table 7). The leaf Fe content ranged between 310 and 460 µg/g depending on genotype (Table 7). The genotypes PI 537632-1038-USA had a leaf Fe content of 460 µg/g which was significantly ($P < 0.05$) higher than leaf Fe content of the genotypes Kiama composite (360 µg/g) and PI 537634-1040-USA (310 µg/g), but was not significantly ($P > 0.05$) different from that of the other genotypes (Table 7). The genotypes Kiama composite, PI 306830-BJ-1632-India, PI 537668-BJ-1085-USA, PI 30441-BJ-2621-Iran, PI 314650-Milutin-114-Kazakistan and PI 407616-BJ-2131-Turkey had statistically ($P > 0.05$) similar leaf Fe contents (Table 7).

4.5.7.2 Whole seed

Safflower genotypes significantly ($P < 0.01$) influenced the Fe content of safflower whole seed (Table 7). The whole seed Fe content ranged between 50 and 80 µg/g (Table 7). The safflower genotype PI 537598-Sina-USA had significantly ($P < 0.01$) higher whole seed Fe content than the genotype PI 537668-BJ-1085-USA, but was not significantly ($P > 0.05$) different from the whole seed Fe contents of other genotypes (Table 7).

4.5.7.3 Cake

The cake Fe content was not significantly ($P > 0.05$) different among the safflower genotypes (Table 7). The Fe content of safflower cake ranged between 70 and 90 µg/g depending on genotype (Table 7).

Table 7. Iron contents of safflower leaf, whole seed and cake

	Iron content (µg/g)		
Genotype	Leaf	Whole seed	Cake
Kiama composite	360b	70ab	70a
PI-537632-1038-USA	460a	60abc	90a
PI-30441-BJ- 2621-IRAN	430ab	60abc	80a
PI-537598-SINA-USA	450a	80a	90a
PI-407616-BJ-2131-TURKEY	390ab	70ab	80a
PI-537634-1040-USA	310b	70ab	80a
PI-537668-BJ-1085-USA	380ab	50c	80a
PI-314650-MILUTIN-114-KAZAKISTAN	390ab	70ab	80a
PI-306830-BJ-1632-INDIA	380ab	70ab	80a
Significance	*	**	NS
LSD	80	20	30

*, **, NS Significant at P = 0.05, 0.01 or non-significant, respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.8 Manganese content

4.5.8.1 Leaf

The leaf manganese (Mn) content of safflower significantly ($P < 0.05$) differed with genotype (Table 8). The leaf Mn content ranged between 280 and 380 µg/g depending on genotype (Table 8). The genotype PI 407616-BJ-2131-Turkey had a leaf Mn content of 380 µg/g which was significantly ($P < 0.05$) higher than the leaf Mn content of the genotypes PI 537632-1038-USA and PI 537598-Sina-USA, but was not significantly ($P > 0.05$) different from the leaf Mn content of other genotypes (Table 8).

4.5.8.2 Whole seed

Safflower genotypes significantly ($P < 0.01$) differed in their whole seed Mn content (Table 8). The whole seed Mn content of safflower seeds ranged between 30 and 50 µg/g depending on genotype (Table 8). The safflower genotypes PI 306830-BJ-1632-India and PI 407616-BJ-2131-Turkey both had a whole seed Mn content of 50 µg/g, which was significantly ($P < 0.01$) higher than the whole seed Mn content of the genotypes PI 314650-Milutin-114-Kazakistan, PI 537668-BJ-1085-USA and PI 30441-BJ-2621-Iran (Table 8). However, the whole seed Mn content of the genotypes PI 306830-BJ-1632-India and PI 407616-BJ-2131-Turkey's Mn did not significantly ($P > 0.05$) differ from the whole seed Mn content of the genotypes PI 537598-Sina-USA, PI 537632-1038-USA, Kiama composite and PI-537634-1040-USA (Table 8).

4.5.8.3 Cake

The cake Mn content of safflower did not significantly ($P > 0.05$) differed with genotype (Table 8). The cake Mn content ranged between 40 and 50 µg/g depending on genotype (Table 8).

Table 8. Manganese content of safflower leaf, whole seed and cake

Genotype	Manganese content (µg/g) Leaf	Whole seed	Cake
Kiama composite	350ab	40abc	40a
PI-537632-1038-USA	300b	40abc	50a
PI-30441-BJ- 2621-IRAN	320ab	30bc	40a
PI-537598-SINA-USA	280b	40abc	40a
PI-407616-BJ-2131-TURKEY	380a	50a	50a
PI-537634-1040-USA	310ab	40ab	40a
PI-537668-BJ-1085-USA	320ab	30bc	40a
PI-314650-MILUTIN-114-KAZAKISTAN	340ab	30bc	40a
PI-306830-BJ-1632-INDIA	340ab	50a	50a
Significance	*	**	NS
LSD	80	10	10

*, **, NS, Significant at P = 0.05, 0.01 and non-significant, respectively. Means with the same letter(s) within column are not significantly different. Means separated using the Least Significant Difference (LSD) at P = 0.05.

4.5.9 Copper content

4.5.9.1 Leaf

Safflower genotypes significantly ($P < 0.05$) influenced the leaf copper (Cu) content (Table 9). The leaf Cu content of safflower ranged between 6.3 and 8.3 µg/g (Table 9). The genotypes PI 537634-1040-USA and PI 306830-BJ-1632-India both had a leaf Cu content of 8.3 µg/g was significantly ($P < 0.05$) higher than the leaf Cu content of the genotypes PI 537668-BJ-1085-USA, PI 537598-Sina-USA, PI 537632-1038-USA and Kiama composite, but not significantly ($P > 0.05$) different from the leaf Cu content of the genotypes PI 314650-Milutin-114-Kazakistan, PI 30441-BJ-2621-Iran and PI 407616-BJ-2131-Turkey (Table 9).

4.5.9.2 Whole seed

The whole seed Cu content of safflower did not significant ($P > 0.05$) differ with genotype (Table 9). The whole seed Cu content of safflower ranged between 140 and 170 µg/g depending on genotype (Table 9).

4.5.9.3 Cake

Similarly, the cake Cu content of safflower was not significantly ($P > 0.05$) influenced by genotype (Table 9). The cake Cu content ranged between 100 and 130 µg/g depending on genotype (Table 9).

Table 9. Copper content of safflower leaf, whole seed and cake

	Copper content (µg/g)		
Genotype	Leaf	Whole seed	Cake
Kiama composite	6.3b	170a	100a
PI-537632-1038-USA	6.3b	160a	110a
PI-30441-BJ- 2621-IRAN	7.6ab	150a	120a
PI-537598-SINA-USA	6.3b	160a	120a
PI-407616-BJ-2131-TURKEY	7.3ab	140a	110a
PI-537634-1040-USA	8.3a	140a	110a
PI-537668-BJ-1085-USA	6.6b	140a	110a
PI-314650-MILUTIN-114-KAZAKISTAN	7.6ab	150a	130a
PI-306830-BJ-1632-INDIA	8.3a	140a	110a
Significance	*	NS	NS
LSD	2.0	40	30

Significance: *0.05. Means with the same letter are not significantly different from each other. Means within columns were separated using the Least Significant Difference at $P = 0.05$.

CHAPTER 5

5.0 DISCUSSION

5.1 Dry matter content

The DM content is used as an indicator of a plant species' resource use strategy, thus its position in a fundamental trade-off between a rapid assimilation and growth at one extreme, and an efficient conservation of resources within well-protected tissues at the other (Wilson *et al.*, 1999; Garnier *et al.*, 2001; Diaz *et al.*, 2004). In the context of livestock feed, DM content in a feed is vital as nutrients such as energy, protein, minerals and vitamins are found in the DM portion of the feed (Reiling, 2011). Dried animal feed with greater than 85% DM content are regarded as high quality feed as they can have a longer shelf-life without molding (Van Saun, 2016).

There were significant ($P < 0.05$) differences in leaf DM and whole seed depending on the safflower genotype and growing season. The leaf DM content ranged between 88.1 and 91.2% and 88.8 and 90.8% in winter and summer grown safflower, respectively, depending on genotype. While, the variation in whole seed dry matter ranged between 94.7 and 96.1% and 91.9 and 94.5% in winter and summer, respectively, depending on genotype. These range of values for leaf and whole seed DM in both winter and summer grown safflower, suggests that the leaves and whole seeds of safflower genotypes used in this study can serve as excellent quality livestock feed for beef and dairy animals (Reiling, 2011; Malakian *et al.*, 2011). The results of the current study are in agreement with results reported by Heuze *et al.* (2012); Malakian *et al.* (2011) and Walker (2006). Heuze *et al.* (2012) researching in the nutritional composition of safflower cake, reported safflower cake obtained by expeller extraction contained 93.2% dry matter. Malakian *et al.* (2011) reported that safflower whole seeds contain

about 94.4% DM. While, Walker (2006) reported that safflower whole seed contained a DM content of 93.0%.

The high leaf DM content in all the safflower genotypes investigated in the current could be beneficial for human consumption as safflower leaves can be used as vegetables. Safflower plants can be used as a vegetable during the early seedling stage (before the start of the elongation phase) (Emongor, 2010, Emongor and Oagile, 2017). Grings *et al.* (2004) reported a value of 92.8% as the leaf DM in safflower leaves.

With exception of the safflower genotype PI 537598-Sina-USA, all the other genotypes had significantly ($P < 0.05$) higher whole seed DM in winter than summer. Winter grown safflower plants accumulated more seed dry matter than summer grown plants because of the longer maturation period of about 138 days after emergence in winter compared to summer with 90 days after emergence (Heggenstaller *et al.*, 2009; Evans *et al.*, 1976). The longer growth period of winter grown safflower implies longer leaf area duration (LAD) than summer grown safflower. There is a positive linear correlation between yield (biomass or dry matter) and LAD in most crops (Heggenstaller *et al.*, 2009; Evans *et al.*, 1976). Leaf area duration which is the integral of leaf area index (LAI) from emergence to physiological maturity significantly determines yield of many crops (Emongor, 2007). The higher plant biomass accumulated by the plants grown in winter than in summer can also be explained by the higher difference in day and night temperature (DIF) in winter. Shang *et al.* (2003) reported that negative DIF significantly reduced shoot and stem dry mass of snapdragon (*Antirrhinum majus* L.). Kedikanetswe (2012) reported that winter grown safflower in Botswana had significantly ($P < 0.05$) higher plant biomass than summer grown safflower.

5.2 Whole seed oil content

Whole seed oil content is a very important economic trait for safflower cultivars and considered one of the most important factors affecting the success of safflower introduction in new areas or regions (Bassil and Kaffka, 2002). The results of this study showed that safflower whole seed oil content significantly ($P < 0.0001$) differed with genotype. The oil seed content of the genotypes varied between 26.1 and 42.2%. The genotype PI 537598-Sina-USA had the highest seed oil content of 42.2% which was significantly ($P < 0.0001$) higher than the seed oil content of all other safflower genotypes under study with exception of PI 537632-1038-USA which had a seed oil content of 41%. Similar results have been reported (Rahamatalla *et al.*, 2001; Arslan and Kucuk, 2005; Gawand *et al.*, 2005; Koutroubas and Papadoska, 2005; Zhang and Chen, 2005; Camas *et al.*, 2007; Abd El-Lattief, 2012; Asghar and Younes, 2015). Rahamatalla *et al.* (2001) reported that safflower oil content depends on cultivar, soil characteristics and climate. The safflower oil content as influenced by genotype reported in some literature as 23.9–40.33% in China (Zhang and Chen, 2005), 26.7–35.8% in Greece (Koutroubas and Papadoska, 2005), 26.3–28.5% (Gawand *et al.*, 2005) and 31.3–36.3% in Turkey (Arslan and Kucuk, 2005). Abd El-Lattief (2012) evaluating 25 safflower genotypes in Egypt reported that safflower oil content varied between 26.4-36.5% depending on genotype. Safflower genotypes 1690 and 1668 had the highest and lowest oil contents of 26.4 and 36.5%, respectively (Abd El-Lattief, 2012). Hamza (2015) in Egypt, reported that six safflower genotypes grown under sandy-saline soil varied in their oil content between 28.5–34.3% depending on genotype. Safflower genotypes are further reported to vary significantly in whole seed oil content (%) and yield per unit area (El-Gayar *et al.*, 1990; Mündel *et al.*, 1999; Omidi, 2006; Camas *et al.*, 2007). While Dajue and Mündel

(1996) reported that safflower whole seeds contained an oil content of between 20–45% depending on genotype.

The primary objective of safflower breeding programs over the years in different parts of the world has been to increase oil content to be 28% per whole seed for safflower to be commercially viable (Sehgal and Raina, 2011). The oil seed content of the safflower genotypes in the current study ranged between 26.1 and 42.2%, with seven genotypes having an oil content ranging between 32 and 42.2%, implying they are commercially viable for vegetable oil seed production. Breeding programs in the United States of America have successfully developed safflower cultivars with oil content of 45–55% (Sehgal and Raina, 2011). From the results of the current study and those reported in literature, it can be concluded that indeed safflower oil content varies with genotype, locality (region), soil type and characteristics and climate. This is in agreement with Singh and Nimbkar (2006), who reported that safflower varieties possess enormous diversity for different traits of economic importance. It is also reported that safflower hybrids with different morphological and physiological characters are available under different macro-climatic conditions (Jonchike *et al.*, 2002). Thus, different safflower accessions or genotypes respond differently depending on the environmental conditions, genotypic characters and management practices (Mehmet, 2016; Ozturk *et al.*, 2008; Singh and Nimbkar, 2006; Jonchike *et al.*, 2002; Rahamatalla *et al.* 2001). Also, Golkar *et al.* (2011) confirmed using molecular markers and safflower seed quality-related traits that significant variation among safflower genotypes for most biochemical traits including oil content, quality and yield.

5.3 Crude protein

The CP is an important indicator of the protein content of a forage crop. Protein content is essential in animal feeding as it is required for maintenance, growth, pregnancy and for

production of milk rich in protein (Saha *et al.*, 2013). An animal feed with greater than 9% CP is considered as high quality feed (Van Saun, 2016). The leaf, whole seed and cake CP content of safflower ranged between 22.1–27.7, 16.3–19.1 and 19.3–22.5%, respectively, depending on genotype and season of growth. Research by Nepal Government (2012) reported a value of 13.5% CP on safflower seeds, which is acceptable basing on the protein requirements of livestock and 2.5% on leaf CP content. However, these results were in disagreement with Grings *et al.* (2004) who reported a high leaf CP of 22.0%. The contradiction can be attributed to harvesting period. Nepal Government (2012) could have used older plants for analysis while Grings *et al.* (2004) used younger plants. Karakaya *et al.* (2004) stated that as a plant matures its protein content decreases due to increased fiber content. However, Grings *et al.* (2004) results were in the range of the current study.

The protein requirement for weaned calves, growing beef animals and finishing is reported to be 13.9, 13.5–15 CP and 12–14% CP, respectively, (NRC, 1996). In Australia, safflower leaves, whole seed and cake are fed to beef and dairy cattle, and sheep as feed supplement or mixed in feed rations (OGTR, 2015). In young sheep, supplementing poor quality diets with safflower cake resulted in increased weight gain and wool growth compared to a barley/urea supplement (OGTR, 2015). Research has shown safflower cake to be a valuable ingredient for dairy cows, with no noticeable effect on flavour or odour of milk produced, but improved the milk and meat quality due to high conjugated linoleic acid (CLA) in the milk and meat (Wood *et al.*, 1999; Griinari and Bauman, 1999; Bottger *et al.*, 2002; Mündel *et al.*, 2004; Scholljegerdes *et al.*, 2004; OGTR, 2015). Meat and milk high in CLA constitutes a health advantage to consumers (Bauman, 1999). Bottger *et al.* (2002) reported that lactating beef cows fed with high-linoleate safflower whole seeds were more capable of maintaining good body condition, whereas cows

fed high-linoleate safflower whole seeds had greater total milk fat. Safflower whole seeds have a beneficial effect on human health, as they are rich in polyunsaturated fatty acids (PUFA), and a source of linoleic acid (0.76 of total FA). Bell *et al.* (2006) reported that addition of safflower oil at 60 g/kg dry matter to dairy cattle feed increased cis-9, trans-11 CLA in milk, which has been suggested to be the best natural source of CLA in the human diet due to its anticarcinogenic properties (Pariza and Hargraves, 1985).

5.4 Neutral detergent fiber

The NDF contains the primary components of the plant cell wall mainly hemicellulose, cellulose and lignin. As cell wall production increases, as it occurs with advancing plant maturity, NDF will increase (Van Saun, 2016; Ball *et al.*, 2017). NDF is used as a measure of feed quality and intake. Animal feed (ruminants) with NDF less than 50% are desirable because as NDF percentages increase, the dry matter intake decreases and chewing activity increases (Van Soest *et al.*, 1991; Van Saun, 2016; Ball *et al.*, 2017). Within a given feed, NDF is a good measure of feed quality and plant maturity (Van Saun, 2016; Ball *et al.*, 2017). Furthermore, an animal feed with less than 50% NDF is considered high quality feed and while feed with greater than 60% NDF is considered as low quality feed (Van Saun, 2016; Ball *et al.*, 2017). Moderate quality feed the NDF content is in the range of 50-60% NDF (Van Saun, 2016; Ball *et al.*, 2017).

In the current study, safflower genotypes significantly ($P < 0.05$) differed in their leaf NDF content, which ranged between 20.5 and 26.2%. This suggested that all the safflower genotypes were of excellent quality and highly consumable because leaf NDF was less than 50% (Van Soest *et al.*, 1991; Van Saun, 2016; Ball *et al.*, 2017). Grings *et al.* (2004) reported that safflower leaves contained less than 49.5% NDF. Safflower is reported as a high quality forage

crop that can be grown in semi-arid and arid regions with limited water resources (Leshem *et al.*, 2000; Bar-Tal *et al.*, 2008) and can also be grown successfully on poor fertile soil and in areas with relatively low temperatures (Koutroubas and Papadoska, 2005).

Regarding the seed NDF, safflower genotypes significantly ($P < 0.05$) differed in their whole seed NDF. The safflower whole seed NDF ranged between 46.0 and 50.3%. This implies that safflower whole seed is a high quality feed for livestock especially for ruminants and all the genotypes used in the study produced seed that can be used to formulate high quality animal feed (Van Soest *et al.*, 1991; Mündel *et al.*, 2004; Heuze *et al.*, 2012; OGTR, 2015; Van Saun, 2016; Ball *et al.*, 2017). Godfrey (2006) reported that safflower whole seeds contained between 34.3 and 50.7% NDF depending on genotype. Malakian *et al.* (2011) and Oguz *et al.* (2007) also reported that safflower whole seeds contained about 40–45% NDF. Recently, Oguz *et al.* (2014) found out that safflower whole seed contained on average about 44.20% NDF. Similar results have also been reported (Dajue and Mündel, 1996). However, the use of safflower whole seed and cake as livestock feed may be limited due to high fiber (NDF) in some genotypes and this reduces feed palatability and digestibility, and safflower whole seed and cake is reported to be deficient in the amino acids lysine, methionine and isoleucine (Heuze *et al.*, 2012; OGTR, 2015). Safflower whole seed can also be used as a protein and energy supplement for beef cattle (Bottger *et al.*, 2002; Scholljerdes *et al.*, 2004; Bolte *et al.*, 2002) and sheep (Kott *et al.*, 2003). Prepartum supplementation with safflower whole seeds high in either linoleic or oleic fatty acids has been reported to increase subsequent conception rates in primiparous beef cows (Lammoglia *et al.*, 1997). Oguz *et al.* (2014) reported that feeding Holstein dairy cows with 2 kg/day of safflower whole seed had no negative effects on milk yield, fat, and serum parameters, but 3 kg/day feed safflower whole seed reduced milk production. However, Dschaak (2009)

demonstrated the use of safflower whole seeds as a means of fat supplementation to lactating dairy cows without negative impact on lactation performance, if added at less than 3% of dietary dry matter.

The safflower cake NDF significantly ($P < 0.05$) differed with genotype. The safflower cake NDF content ranged between 54.6 and 61.2%, depending on genotype. This suggested that the safflower cake was of medium quality for livestock feed (Van Soest *et al.*, 1991; Van Saun, 2016; Ball *et al.*, 2017). The hull is the main source of fiber in the seed, so the level of crude fiber in safflower cake varies depending on the level of hulls remaining. Fiber can be 30% to 40% in undecorticated cakes and as low as 10% in decorticated cakes (Dajue and Mündel, 1996). The safflower cake from dehulled seeds can be used in compound feeds for pigs and poultry as a source for supplemental amino acids lysine, methionine and cysteine (Voicu *et al.*, 2009). The safflower cake with high fiber content can be used for ruminant feed (Voicu *et al.*, 2009). The variation in the chemical composition of safflower cake depends on the amount of hulls and on the extent of oil extraction (Heuze *et al.*, 2012). In the current study the safflower cake after oil extraction was obtained from hulled safflower seeds and the oil extraction efficiency was 95% hence explaining the medium NDF content of 54.6 to 61.2%. Dixon *et al.* (2003) reported that the cake NDF of safflower whole seed after oil extraction was 63.4% and was suitable as sheep feed. NRC (2001) reported that safflower cake contained 54.0% NDF. Decorticated (dehulled) safflower cake can be used as a protein supplement for low protein forages in livestock diets or in poultry back grounding diets (Peiretti, 2009).

5.5 Acid detergent fiber

Acid detergent fiber is a subset of NDF. Acid detergent fiber contains the poorly digestible cell wall components mainly cellulose, lignin, silica, ash and insoluble forms of nitrogen but not

including hemicellulose (Van Saun, 2016; Ball *et al.*, 2017). ADF is used to calculate digestibility or net energy of feed. Similarly as in NDF, low levels of ADF are recommended because at high ADF levels digestibility of animal feed decreases (Saha *et al.*, 2013). The objective is to have an ADF value of less than 35 in either legume or grass forages (Van Saun, 2016; Ball *et al.*, 2017). In the current study, the leaf, whole seed and cake ADF ranged between 26.5–32.7, 39.7–48.0 and 45.0–50.7%, respectively, depending on safflower genotype. The results implied that safflower leaves are more digestible as compared to safflower whole seeds and cake, hence most potential livestock feed. However, Godfrey (2006), Malakian *et al.* (2011) and Oguz *et al.* (2014) confirmed that safflower whole seeds contain less than 35% ADF. Godfrey (2006) reported that safflower whole seeds contained about 28.9 ± 5.0% ADF. While, Malakian *et al.* (2011) reported that safflower whole seeds contained between 30–32% ADF. Oguz *et al.* (2014) reported that safflower whole seed contained approximately 32.52% ADF. Walker (2006) reported that safflower whole seeds contained about 40.0% ADF and concluded that fibers cannot be used solely to qualify an animal feed, but other variables such as CP, mineral content and DM should be considered also. Alobeid *et al.* (2010) affirmed that safflower cake with high protein of 25% had moderate fiber content of 50% ADF.

5.6 Acid detergent lignin

Acid detergent lignin measures the amount of lignin contained in an animal feed. Lignin is indigestible and hence has a negative impact on cellulose digestibility. As lignin content in a feed increases, digestibility of cellulose decreases, thereby lowering the amount of energy potentially available to the animal (Saha *et al.*, 2013). Moreover, at high ADL, NDF and ADF contents in animal feed, feed intake and digestibility, plus and animal production and performance decreases (Van Saun, 2016; Ball *et al.*, 2017). In the current study, the leaf, whole seed and cake ADL

ranged between 6.71–0.7%, 14.0–20.7% and 18.0– 20.8%, respectively, depending on safflower genotype. The results indicated that indicated that safflower leaves and whole seeds have high feed intake and digestibility quality as compared to safflower cake due to low ADL values. Grings *et al.* (2004) reported that mature safflower leaves contained 36.0% ADL. While, Dixon *et al.* (2003) reported that safflower cake contained an ADL value of 15.5%. The difference in Grings *et al.* (2004) and Dixon *et al.* (2003) results could be attributed to stage of maturity of samples at harvest. More matured plants samples yields high NDF, ADF and ADL values due to increased fibrous components (Parissi *et al.*, 2005), hence the high value of Grings *et al.* (2004).

5.7 Ash content

Ash is the total mineral content of a forage or diet or animal feed (Van Saun, 2016; Ball *et al.*, 2017). The ash does not separate out any individual minerals and does not separate macro and micro-minerals of interest from silica and other less important minerals. It is important to understand what constitutes the normal ash content of a feed, because if ash content is abnormally high, there is a high possibility that the feed/forage is contaminated with soil which is undesirable (Hoffman and Tayson, 2005). The ash content of a seed/grain is expected to be within the range 1-4% on DM basis, for it to be rated as a quality animal feed (Schroeder, 2012; Van Saun, 2016). In the current study, the leaf, whole seed and cake ash contents ranged between 0.83 and 1.13%, 0.95 and 1.41%, and 1.10 and 1.6% on DM basis, respectively, depending on safflower genotype, which was within the normal range for animal feed (Schroeder, 2012; Van Saun, 2016). Rahamatalla *et al.* (1998) reported that safflower whole seeds contained 2.30-5.54% ash content. While, Nagaraj (2009) reported that safflower whole seed had an ash content of 2.10-3.50% ash content. Malakian *et al.* (2011) further reported that

safflower whole seed contained an ash content of 2.10% on DM basis. The results of the current study confirm that safflower leaves, whole seed and cake are excellent livestock feed.

5.8 Effect of season on feed quality

In general, all variables (DM, NDF, ADF, ADL and ash) used to measure feed quality for livestock were higher in safflower grown in winter than in summer, with exception of CP (winter 17.4% and summer 17.6%). This was attributed to the cool growing season in winter making the safflower plants to mature after a longer time (138 days) than summer (90 days) grown safflower. Moatshe *et al.* (2016) reported that in Botswana safflower grown in winter matured after 145 days compared to 110 days when grown in summer. Kedikanetswe (2012) and Emongor *et al.* (2013) reported that safflower grown in winter and summer matured after 138 and 116 days after germination respectively. The difference in maturation duration was attributed to high temperatures in summer than in winter (Kedikanetswe, 2012, Emongor *et al.*, 2013; Moatshe *et al.*, 2016; Emongor and Oagile, 2017). Temperature has a critical impact on safflower plant phenology especially during vegetative stage (Ritche and Ne Smith, 1991; Goudriaan and Laar, 1994; Shabana *et al.*, 2013; Rasul *et al.*, 2016; Emongor and Oagile 2017). Temperature also interacts with photoperiod and vernalisation at the earlier phases of safflower phenology (Porter and Decolle, 1988; Emongor and Oagile, 2017). Cell wall production increases with advancing or long plant maturity, therefore, plant DM, NDF, ADF and ADL increases (Parissi *et al.*, 2005; Van Saun, 2016; Ball *et al.*, 2017).

5.9 Mineral nutrient contents

A feedstuff with proper mineral supply is the key to successful animal production. Minerals make up a small portion of an animal diet. However, they play an important role in health, growth and reproduction. It is also important to note that animal mineral requirements cannot be

considered independently as minerals work hand in hand (Ward, 2005). The macro-minerals (calcium, phosphorus, magnesium, potassium, sodium and sulphur) and micro minerals (iron, copper, zinc, manganese and molybdenum) are of importance to livestock production (Ward, 2005; Van Saun, 2016).

5.9.1 Phosphorus content

Phosphorus (P) is one of the important minerals in animal nutrition. It is the second most abundant element in an animal body after calcium (Ca). Phosphorus creates the bone structure of animals and 80% P is stored in the bones (Steward, 2007; Datnoff and Wade, 2007). P is also a co-factor of many enzymes and activates the vitamin B complex. For this process to be effective, P and Ca ratio should be balanced. The optimum Ca: P ratio should be approximately 1.5 : 1 with the range of 1 : 1 to 1 : 4 being satisfactory (Steward, 2007; Ward, 2005). The P requirement in livestock is between 0.25–0.30%, thus any feed providing this P value is regarded as a quality animal feed (NRC, 1996). In the current study, the leaf, whole seed and cake P contents ranged between 3.31–4.95, 5.47–7.87 and 6.98–7.90 mg/g, respectively, depending on safflower genotype. The leaf (0.33–0.5%), whole seed (0.55–0.79%) and cake (0.7–0.79%) P contents of safflower are within the range of P requirement of 0.25–0.30 % for a feed to qualify as high quality (NRC, 1996). These results showed that safflower leaves, whole seeds and cake are high quality feed based on their P contents. Ibrahim and Hasan (2005) reported that safflower whole seeds and leaves contained 0.6 and 0.3% P, respectively. Nepal Government (2012) reported that safflower whole seeds and leaves contained 323 and 35 mg/100g P, respectively. Safflower whole seeds and cake are also reported to contain 638 and 181.19 mg/100g P contents (United States Department of Health Services (USDH), 2010). While, Heuze *et al.* (2012) reported that safflower cake contained 0.67% P. As oil is extracted from the safflower seeds, the residue (cake)

that remains becomes concentrated, hence high mineral contents explaining the higher minerals in the cake or than whole seed (Walker, 2006).

5.9.2 Potassium content

Animals need potassium in large amounts to maintain normal body and organ function. Potassium (K) works in conjunction with sodium (Na) to transport nutrients in and out of the cells. It also maintains health by controlling cellular water balance (Ward, 2005). Farm animals generally require 0.6–0.7% K, with a maximum tolerable of 3.0% K (DM basis) in their ration. Most forage sources are relatively high as they can provide 1–2% K whereas cereal grains are low 0.30–0.36% K. Oil meals can also provide more K, for example canola meal provides 1.35% K (Wickramasuriya *et al.*, 2015). In the current study safflower leaves, whole seeds and cake contained between 5.61–6.62, 0.844–1.23, 1.07– 1.29% K, respectively, depending on genotype. These results showed that safflower leaves, whole seeds and cake are excellent sources of K for animal feed (Ward, 2005; Heunze *et al.*, 2016). Ibrahim and Hasan (2005) reported that safflower whole seeds and leaves contained 0.76% and 4.37% K, respectively. While, USDH (2010) reported that safflower whole seed and cake contained 0.019 and 6.8% K, respectively.

5.9.3 Calcium content

Calcium (Ca) is one of the most important mineral elements in animal bodies. It comes first before P, and as earlier mentioned, the two work dependently. Most of body calcium (99.0%) is stored in the bones and teeth (Steward, 2007). Animals need the correct amount of Ca for the nervous and muscular systems to function properly (Steward, 2007). The required Ca contents for growing, gestating and lactating cows are 0.31, 0.18 and 0.27%, respectively, based on stage of production. General Ca requirement of livestock was reported at a range of 0.50–0.60%

(NRC, 2000). The maximum tolerable Ca content is 1.80% in feed rations (NRC, 2000). In the current study, safflower leaves, whole seeds and cake contained 1.06–1.65, 0.95–1.13 and 0.88–1.08% Ca, respectively, depending on genotype. Again the results of the current study confirmed that safflower leaves, whole seeds and cake are excellent sources of Ca for livestock (NRC, 2000, Ward, 2005). Safflower leaves, whole seed and meal can be used in feed ration formulations for various livestock or can be used as feed supplement. According to USDH (2010), safflower whole seed and cake contained 0.022% Ca. The Nepal Government (2012) reported that safflower whole seeds and leaves contained 0.24 and 0.19% Ca, respectively. While, Cosge *et al.* (2007) reported that safflower leaves contained 0.018% Ca. The differences in mineral element content in various parts of safflower reported in literature and the results of the current study was attributed to the differences in soils and climate in various regions where safflower grows (Marschner, 2005).

5.9.4 Magnesium content

Magnesium (Mg) is a key component in initiation of many metabolic enzymes and pathways in animals (Ward, 2005). It is also important in neuromuscular function of animals. Deficiencies in Mg content can result in reduced calving rate, calf vigor and rate of gain (Ward, 2005). Low Mg content in the feed does not affect the calf performance directly but it affects cow's milk production (Ward, 2005). The magnesium requirement of beef cattle from growing calves, dry cows and lactating cows were reported as 0.20, 0.12 and 0.10% respectively (NRC, 1996). The maximum tolerable is 4.0% Mg for enhanced growth. Mature sheep also require 0.11% Mg for normal growth and milk production (Wand, 2010). In the current study, safflower leaves, whole seed and cake contained 0.39–0.49, 0.44–0.56 and 0.45–0.5% Mg, respectively, depending on genotype. The results of the current study showed that safflower leaves, whole seeds and cake

are excellent sources of Mg to be used as livestock feed. USDH (2010) reported that safflower whole seed and cake contained 0.099 and 0.35% Mg, respectively. While Heuze *et al.* (2012) and Smith (1996) reported that safflower cake contained 0.28 and 0.31% Mg, respectively. Canola cake was reported to contain 0.57% Mg (Wickramasuriya *et al.*, 2015).

5.9.5 Sodium content

Sodium (Na) is responsible for the proper function of the nervous and muscular systems (Ward, 2005; Steward, 2007). Sodium works in conjunction with chlorine (Cl) to regulate body pH and the amount of water retained in the body. A deficiency of Na causes loss of appetite and inefficient weight gains. Sodium is commonly deficient in livestock diets, but Cl levels are usually adequate (Steward, 2007). The sodium requirement of cattle is 0.07% for growing calves and dry cows, and 0.10% for lactating cows (NRC, 2000). The maximum recommended Na is 4.0% (NRC, 2000). Goats and sheep needs 1.5 g/day and 0.06% Na contents, respectively (Wand, 2010). In the current study, safflower leaves, whole seeds and cake contained 0.51–0.69 0.32–0.36, 0.42–0.37%, respectively. These results showed that safflower leaves, whole seeds and cake are excellent sources for Na to be used in livestock feeds and in feed ration formulation (NRC, 2000; Ward, 2005). The results of current study also showed that safflower cake and whole seed can replace canola, sunflower and soy meal in livestock feed ration formulation (Smith, 1996; Weiss, 2000; Heuze *et al.*, 2012). USDH (2010) reported that safflower whole seed and cake contained 0.01–0.03% Na.

5.9.6 Zinc content

Zinc (Zn) plays an important role in immune response, enzyme systems and hoof health in animals (Ward, 2005). It also plays an important role in DNA, RNA and protein production.

Because of this wide scope and body demand, it is required at 30 ppm in cattle feeding (Ward, 2005). Exact amount are not known for goat requirements, but between 10–60 ppm is considered satisfactory (NRC, 2000). Sheep require 36 ppm Zn in their diet (Wand, 2010). However, Zn levels should not exceed 1000 ppm or it will affect performance and toxicity can develop (Ward, 2005). In the current study, safflower leaves, whole seed and cake contained 70– 90, 90–120 and 90–120 ppm Zn, respectively, depending on genotype. This implies that safflower leaves, whole seeds and cake are excellent sources of Zn for animal feed. USDH (2010) reported that safflower whole seeds and cake to contain 50 ppm of Zn.

5.9.7 Iron content

Iron is primarily required for haemoglobin formation. Deficiency symptoms include anaemia, depressed immunity and loss of weight. Iron deficiency is rarely observed in grazing cattle (Wand, 2010). Fe requirements of livestock are 50 ppm for cattle, 30–75 ppm for sheep and goats. A daily intake of 750 ppm is considered acceptable for lactating goats (Wand, 2010; NRC, 2000). In the current study, safflower leaves, whole seeds and cake contained 310–460, 50–80, 70–90 ppm Fe, respectively, depending on genotype. Nepal Government (2012) reported that safflower whole seeds and leaves contained 46 and 570 ppm Fe, respectively. While, NRC (1996) reported that safflower whole seeds and cake contained 58.6 and 50 ppm Fe, respectively. The results of the current study confirm that safflower leaves, whole seed and cake are excellent sources of Fe for livestock feed.

5.9.8 Manganese content

Manganese (Mn) plays an important role in growth and reproduction of animals (Ward, 2005). Manganese is linked to growth through its involvement in specific enzyme functions related to

skeletal cartilage (Ward, 2005). Furthermore, as dietary levels increase, the concentration of Mn increases in reproductive tissues, offering a direct link between Mn and fertility (Smith, 1996; Ward, 2005). Requirements of Mn may vary depending on the stage of production. For example, growing and finishing cattle require 20 ppm in their diet, while lactating cows require 40 ppm. The maximum tolerance can be as high as 1000 ppm before it causes noticeable negative effect (Ward, 2005). Sheep require 20–40 ppm of Mn in their diets (Wand, 2010). While the optimum quantity of Mn for poultry (chicken and turkeys) is about 50 ppm (Titus, 1934; Smith, 1996; Weiss, 2000).

In the current study, safflower leaves, whole seed and cake contained 280–380, 30–50 and 40–50 ppm Mn, respectively, depending on genotype. USDH (2010) reported that safflower whole seeds and seed cake to contained 20 ppm Mn. While, Smith (1996) reported that safflower meal contained 20.4 ppm Mn. Based on the results of the current study, safflower leaves, whole seeds and cake are excellent sources of Mn for livestock feed.

5.9.9 Copper content

Importance of copper (Cu) includes; red cell health, collagen development, reproduction and immunity (Ward, 2005). Copper works in conjunction with molybdenum (Mo) and inorganic sulphur to create enzymes involved in nucleotides and vitamin metabolism. Copper toxicities can occur in cattle when dietary levels exceeding100 ppm (Ward, 2005). Toxicities are more common in sheep as they occur at 25 ppm, particularly when Cu : Mo ratio is greater than 10 : 1, thus the Cu : Mo ratio should be maintained at 5 : 1 and 10 : 1 (Ward, 2005; Pugh, 2016). In the current study, safflower leaves, whole seeds and cake contained 6.3–8.3, 140–170 and 100–130 ppm Cu, respectively, depending on genotype. USDH (2010) reported that safflower seeds

contained 17.3 ppm Cu. The results if the current study showed that safflower leaves, whole seeds and cake are excellent sources of macro- and micro minerals need for livestock nutrition.

CHAPTER 6

CONCLUSSION

Based on the oil and the nutritive contents (DM, CP, NDF, ADF, ADL, macro-and micro minerals) of leaves, whole seeds and cake (cake after oil extraction) of the nine safflower genotypes, it was concluded that safflower leaves, whole seeds and cake after oil extraction are excellent sources of nutrients for livestock feed especially ruminants and poultry; and it can be used for feed formulation and supplementation.

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