Dairy goats fed sunflower hay intercropped with chickpea in small-scale systems. Part I: Animal performance

Cabras lecheras alimentadas con heno de girasol intercalado con garbanzo en sistemas de pequeña escala. Parte I: Desempeño animal

Cabras leiteiras alimentadas com feno de girasol consorciado com grão de bico em sistemas de pequena escala. Parte I: Desempenho dos animais

Aurora Sainz-Ramírez; Julieta-Gertrudis Estrada-Flores; José Velarde-Guillén; Felipe López-González; Carlos-Manuel Arriaga-Jordán*.

Instituto de Ciencias Agropecuarias y Rurales (ICAR), Universidad Autónoma del Estado de México, Campus UAEM El Cerrillo.

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***Corresponding author.** El Cerrillo Piedras Blancas, 50090 Toluca, Estado de México, México. Tel: +52 722 481 16 07. E-mail: cmarriagaj@uamex.mx

Abstract

Background: Small-scale livestock systems have an important role in providing quality foods for a worldwide growing demand for animal products, to improve rural livelihoods, and to reduce the environmental footprint. There is a need to increase productivity through improved feeding strategies. Sunflower is native of México and chickpea is a common crop in the study area, that may represent an alternative to improve feeding of dairy goats. **Objective:** To evaluate through participatory on-farm research the effect of substituting maize straw traditionally used for feeding dairy goats with a better-quality forage adapted to the region, but not used for dairy goats, on the yield and chemical composition of milk of goats. **Methods:** Twenty-eight multiparous Saanen dairy goats were randomly assigned to two treatments (14 goats each), in a 30 day on farm experiment on a small-scale farm. Two weeks prior to the experiment all goats received an adaptation diet. One treatment (MZST) received the conventional diet of lucerne hay (200 g/goat/day) and concentrate (400 g/goat/day), plus 600 g/goat/day (50% of the ration) of maize straw. The second treatment (SFCPT) contained the same lucerne and concentrate content but with 600 g/goat/day of the sunflower-chickpea hay. Milk yield and composition, and the live weight and body condition of the goats were recorded for each treatment. **Results:** SFCPT significantly increased the milk yield, protein, and solids content, but there was no difference in milk fat content. **Conclusion:** The treatment with sunflower and chickpea hay increased milk production, protein content and total solids.

Keywords: *alternative forages; caprine; chemical composition; chickpea; feed; goats; hay; legumes; Mexico; milk; sunflower*

Resumen

Antecedentes: Los sistemas de producción animal en pequeña escala tienen un papel importante para proveer de alimentos de calidad para una demanda creciente, para mejorar la calidad de vida rural, y reducir la huella ambiental. Existe la necesidad de incrementar la productividad a través de estrategias de alimentación mejoradas. El girasol es originario de México y el garbanzo es un cultivo común en la zona de estudio que pueden representar una alternativa para la alimentación de cabras lecheras. **Objetivo:** Evaluar mediante investigación participativa en finca el efecto de sustituir la paja de maíz utilizada tradicionalmente para la alimentación de cabras lecheras con un forraje de mejor calidad adaptado a la región, pero no utilizado en la alimentación de cabras lecheras, en términos de rendimiento y composición química de leche de cabras. **Métodos:** Veintiocho cabras multíparas lecheras Saanen fueron asignadas aleatoriamente a dos tratamientos (14 cabras a cada uno) en un experimento de 30 días en una finca a pequeña escala. Dos semanas antes del experimento todas las cabras recibieron una dieta de adaptación. El tratamiento (MZST) recibió la dieta convencional de heno de alfalfa (200g/cabra/día) y concentrado (400 g/cabra/día), más 600 g/cabra/día (50% de la ración) de paja de maíz. El segundo tratamiento (SFCPT) tuvo el mismo contenido de alfalfa y concentrado, pero con 600 g/cabra/día de heno de girasol-garbanzo. Se registraron los rendimientos y composición de leche, peso vivo y condición corporal de las cabras de cada tratamiento. **Resultados:** SFCPT incrementó significativamente el rendimiento de leche, y contenido de proteína y sólidos, pero no hubo diferencias en contenido de grasa. **Conclusión:** El tratamiento con heno de girasol y garbanzo incrementó la producción de leche, el contenido de proteína y sólidos totales.

Palabras clave: *alimento; cabras; caprinos; composición química; forrajes alternativos; heno; girasol; garbanzo; leche; leguminosa; México*.

Resumo

Antecedentes: Os sistemas de produção animal em pequena escala têm um papel importante no fornecimento de alimentos de qualidade para atender uma demanda mundial crescente desses produtos, para melhorar a subsistência rural e para reduzir a pegada ambiental. Há uma necessidade, porem, de aumentar a produtividade através de estratégias de alimentação melhoradas. O girassol é nativo do México e o grão-de-bico é uma cultura comum na área de estudo que podem representar uma alternativa na alimentação de caprinos leiteiros. **Objectivo**: Avaliar através de investigação participativa na exploração o efeito da substituição da palha de milho tradicionalmente utilizada na alimentação de cabras leiteiras por uma forragem alternativa adaptada à região, mas não utilizada até o momento, em termos de rendimento, composição química de leite fresco de cabras. **Métodos**: Vinte e oito cabras leiteiras Saanen multipares foram designadas aleatoriamente a dois tratamentos (14 cabras/tratamento), em um experimento de 30 dias numa fazenda de pequena escala. Duas semanas antes do experimento, todos os caprinos receberam uma dieta de adaptação. Um tratamento (MZST) recebeu a dieta convencional de feno de alfalfa (200 g/caprino/dia) e concentrado (400 g/caprino/dia) mais 600 g/caprino/dia (50% da ração) de palha de milho. O segundo tratamento (SFCPT) tinha a mesma proporção de alfalfa e concentrado, mas com 600 g/caprino/dia de feno de girassol e grão-de-bico. O rendimento e composição do leite, o peso vivo e a condição corporal das cabras foram registrados de cada tratamento. **Resultados**: SFCPT aumentou significativamente o rendimento do leite e o conteúdo de proteínas e sólidos, mas não houve diferença no conteúdo de gordura láctea. **Conclusão**: O tratamento com girassol e feno de grão de bico aumentou a produção de leite, o teor de proteína e os sólidos totais.

Palavras-chave: *bode; cabras; comida; composição química; feno; forragens alternativas; girassol; grão de bico; leite; leguminosas; México.*

Introduction

Small-scale livestock systems ameliorate rural poverty (FAO, 2010), but must adapt the feeding strategies of their herds and flocks to new scenarios in the face of current challenges (Shikuku *et al*. 2016). Goat systems may lead to sustainable rural livelihoods (Daskiran *et al*. 2018), but most have low productivity due to low quality and availability of feeds, low genetic merit of stock, and in dairy production, low milk yields (Souza *et al*. 2017). Improving goat rations can enhance productivity, identified as a necessary move to improve livelihoods (Makkar, 2016). Quality forages may have positive impacts on milk yields (Cabral *et al*. 2015) and, if home-grown, improve efficiency in resource use (Rao *et al*. 2015).

The association of legumes with grasses or other forage plants has environmental, agronomic and economic benefits, as legumes improve soil fertility through nitrogen fixation, promotes soil conservation, reduces weed invasion, improves yields, and enhances the protein content of forages (Maxin *et al*., 2016). Therefore, research in forage production with locally adapted legumes and other forage plants is warranted.

Mexico is the 12th largest goat milk producer country, where 68% of goat farms are extensive or semi-extensive, and 32% are intensive farms (SIAP, 2020). The feeding of goats in extensive or semi-extensive farms is based on grazing of natural grasslands and shrub browsing, while feeding in intensive systems is based on lucerne, concentrates, and crop residues such as maize straw (Salinas *et al*. 1999; Fuentes *et al*. 2001).

Maize straw is high in fiber and low in digestibility and protein (Fuentes *et al*. 2001). On the other hand, sunflower (*Heliantus annus*) is an ingredient rich in lipids (Rodrigues-Gandra *et al*. 2017) and has been used to increase the fat content of diets, which in goats has resulted in increased milk protein content (Sanz-Sampelayo *et al*. 2007). Sunflower is native of México, and as a crop, is adapted to different climates in the country.

Chickpea (*Cicer arietinum*) is a legume cultivated mostly for its pulse-grain for human consumption (Herrera-Flores *et al*. 2019). As a legume, chickpea fixes atmospheric nitrogen, improving soil fertility. Mexico is the third largest producing country in the world, and the study area of the work herein reported is the fourth producer in the country. Both sunflower and chickpea are not traditionally used as feed for goats, but could represent an alternative forage option to improve the diet quality and productivity of dairy goats.

Therefore, the objective was to evaluate through participatory on-farm research the effect of substituting maize straw traditionally used for feeding dairy goats with a better-quality forage based on sunflower and chickpea hay adapted to the region, but not used for dairy goats, on the yield and chemical composition of milk of goats.

Materials and methods

Ethical considerations

Experimental procedures with dairy goats and work with the collaborating farmer followed guidelines accepted by the Instituto de Ciencias Agropecuarias y Rurales (ICAR) of Universidad Autónoma del Estado de México, and institutionally approved (DICARN-1319).

Work followed an adaptive participatory rural research approach, methodology well validated in different countries (Kraaijvanger and Veldkamp 2015; Flor *et al*. 2017); where the main goal is to find solutions to problems faced by farmers through collaborative work between farmers and researchers promoting innovation in the utilisation of local resources (Hauser *et al*. 2016; Aare *et al.* 2021).

Location of the study

An on-farm experiment, following the participatory livestock technology development approach, was performed in Yuriria, state of Guanajuato 20° 12' 51" N and 100° 08' 19" W in central Mexico. The region is a plateau with a mean altitude of 2000 masl, a semi-warm sub-humid climate with rains in summer and a dry season in winter, and a mean rainfall of 600-700 mm/year.

Sunflower-chickpea hay

The sunflower intercropped with chickpea was sown after a maize crop on 1.0 ha on September 2nd 2018, towards the end of the rainy season and harvested on December 20th at 109 days post sowing. Sowing rate was 10 kg seed/ha (estimated density of 80,000 plants/ha) of sunflower (cv. Tiacaque) and 75 kg/ha (90,000 plants/ha) of a local landrace chickpea seed. Distance between rows was 80 cm, whilst distance between plants was 15 cm. At harvest, the sunflower was at the R8 phenological stage (Schneiter and Miller, 1981) and chickpea at the R7 stage (Herrera-Flores *et al*., 201), and both were left to dry in the field (for 96 h), after which all material was ground through a 2.5 cm sieve with a tractor-driven hammer mill. The final proportion of each forage was 60 sunflower and 40% chickpea. Crop yield was approximately 7.5 tonnes DM of field-cured dried sunflower-chickpea hay (SFCP).

Animals

The experiment was performed with 28 Saanen multiparous dairy goats, divided randomly into two groups of 14. Goats were in late lactation (195±11 days in milk). Other authors have undertaken feeding experiments with late lactation goats (Madsen *et al.* 2005; Russo *et al,* 2013; Muklada *et al*. 2018).

Pre-experimental live weight was 58±3.2 kg, daily milk yield of 1.2±0.2 kg, and body condition score (BCS) of 6 on a scale from 1 (very thin) to 9 (very fat) (Aumont *et al*. 1994). An analysis of variance was performed on goat live weights before the initiation of the experiment to determine if there were significant differences between the two goat groups. There were no differences and therefore the completely randomized design was deemed adequate.

The goats were housed in open pens (one pen per treatment group), with dirt floor and roofed in one third of the area, covering above the feeding troughs, which ran the whole width of the pen. The feeding area had a 1.0 m concrete floor along the feeding troughs. Each pen was 7.5 x 8.0 m, providing ample space for each goat group (4.3 m^2/goat).

The forage treatments and lucerne hay were fed to each treatment group collectively, and the concentrate supplement fed individually to each goat at milking. Goats were milked by hand once a day at 7:00 h.

Two weeks prior to the commencement of the experiment, all goats in the flock received an adaptation diet that included the concentrate supplement used in the experiment at 400 g/goat/day, and 200 g/goat/day of lucerne hay, and 600 g/goat/day of a 50:50 (fresh basis) mixture of maize straw and sunflower-chickpea hay, plus minerals and water *ad libitum*.

The experimental treatments MZST or SFCPT were implemented in each of the experimental groups at the beginning of the trial. Milk yield for each goat was recorded daily, and a 20 ml daily milk sample from each goat was refrigerated (3 °C), with a weekly pooled sample analyzed for milk composition. Mean values were used for analyses.

Live weight (LW in kg) was recorded on the first and last day of the experiment with a hook digital scale, and body condition score (BCS) was assessed simultaneously by the same observer in both weight recordings.

The experiment took place from June 10th to July 9th, 2019 (30 days). The length of the experiment was decided in consultation with the participating farmer taking into consideration his flock management needs as well as sufficient time to evaluate the effect of the experimental feeding treatments. The response to a diet by dairy ruminants can be observed after a few days of the diet introduction, and short experimental periods in feeding experiments for dairy goats are well validated and accepted in the scientific literature, as demonstrated by Charpentier *et al*. (2019). Thus, a 30 day experiment was of an appropriate duration to detect effects due to the two feeding treatments in the performance of milking dairy goats.

Diets

Treatment MZST, the conventional diet (control treatment) was made by concentrate, lucerne hay, and maize straw; whilst the experimental treatment diet (SFCPT) was made by concentrate, lucerne hay, and the sunflower-chickpea hay in place of the maize straw. The forages (lucerne hay, maize straw and sunflower-chickpea hay) were ground with a hammer mill through a 2.54 cm screen.

Diets provided a 67% forage (lucerne hay plus maize straw or sunflower-chickpea hay): 33% concentrate ratio, with water, a commercial mineral and vitamin mix for goats provided *ad libitum*.

The concentrate supplement for the experiment was a homemade mix of commercial compound concentrate for dairy goats with 22% CP (65% fresh weight), ground white maize grain (20% fresh weight), and ground sorghum grain (15% fresh weight), with a final CP content for the mixture of 18%.

Table 1. Composition of treatment rations on a dry weight basis (g DM/goat/d).

Ingredient	**MZST**	**SFCPT**
Concentrate	400	400
Lucerne hay (ground)	200	200
Maize straw (ground)	600	0
Sunflower – chickpea hay (ground)	0	600
Total	1,200	1,200

DM: Dry mater; MZST: concentrate+lucerne hay+maize straw; SFCPT: concentrate+lucerne hay+sunflower-chickpea hay.

The concentrate was provided individually to each goat at milking, and the ground lucerne hay and experimental forages (maize straw or sunflower-chickpea hay) were thoroughly mixed before allocation to the goats to minimize selection and offered collectively to each experimental group of goats (Table 1), offering half of the forage ration in the morning and the other half in the afternoon. Every morning, before a new allocation of forages, refusals were collected, weighed, and a sample taken for determination of the dry matter content thereafter.

Samples of the experimental diets (forage plus concentrate) were taken daily before being allocated, and samples of the individual ingredients taken weekly for chemical analyses.

Both treatments met the requirements for energy and protein of milking goats (NRC, 2007). However, the diets were not isoproteic nor isoenergetic since the objective of the work was not to compare the two forage sources (MZST or SFCP) on a nutritional basis. The objective of this work was in line with what Rao *et al.* (2015) described as the need to improve feeding strategies for small-scale livestock systems, based on quality forages to increase their productivity to better contribute to sustainable livelihoods and to meet world challenges for livestock production.

Chemical composition of feeds

Samples were dried at 55 °C for 72 hours in a draught oven and then milled through a 1 mm sieve. Samples of all feeds were analysed for ash by incineration at 550 °C (AOAC, 1990) to determine organic matter (OM), crude protein (CP) by the Kjeldahl method (N x 6.25), and ether extract (EE) following AOAC (1990) procedures. Neutral detergent fiber (NDF), and acid detergent fiber (ADF) that follows Van Soest *et al*. (1991). *In vitro* dry matter digestibility (IVDMD) followed the method of Tilley and Terry (1963) with rumen fluid obtained through a stomach tube from five donor female goats.

Milk sampling and analyses

Milk yield for each goat was recorded daily. A 20 ml sample of milk from each goat was taken daily, pooled by treatment, and refrigerated (3 °C) before being analysed for milk composition, determined with an automatic ultra-sound milk analyzer (Lactoscan MCC).

Samples were pooled by treatment due to restraints in the on-farm adaptive experiment with the collaborating farmer as the milk analyzer was facilitated by the farmers' association only once per week.

This illustrates one of the limitations of the adaptive research approach followed in this experiment, with the goal of adapting a given technology to local conditions through experiments in the farms (Flor *et al*. 2017).

Adaptive research not only applies existing knowledge, but the research with farmers investigates how results adapt to the farmers' objectives and productive conditions to facilitate dissemination and adoption of results (Stroup *et al*. 1993).

Compared to experimental centers, on-farm research with small-scale farmers faces limitations like small land holdings, small herds or flocks, and management constraints to carry out the experiments, but these are offset by the benefits of participatory research as stated by Stroup *et al*. (1993).

Statistical analyses

Variables for goat performance and chemical composition of milk were analyzed with ANOVA (Minitab 14 statistical software) following a completely randomized design with the following model:

$$Y_{ij}= \mu_i + t_j \, e_{ij} \qquad (1)$$

Where μ= general mean, t= effect of treatments (i = 1, 2) and e= residual variation. Analyses for variables were on the mean values. Significant differences were declared at $p \leq 0.05$.

Results

Chemical composition of feeds

The sunflower-chickpea hay, by combining a legume and an oil plant, resulted in a forage high in crude protein content and with a high ether extract (lipid) (Table 2). The total experimental diet (SFCPT) was thus 33% higher in CP, 75% higher in EE and 15% higher in IVDMD than the control diet (MZST).

Table 2. Chemical composition of feeds and treatment rations (MZST and SFCPT) (g/Kg MS).

	DM	**OM**	**CP**	**NDF**	**ADF**	**IVDMD**	**EE**
CON	967.07	903.25	179.58	285.12	79.21	827.20	20.00
LH	880.14	900.15	180.47	360.61	280.52	670.41	22.51
MS	894.14	933.59	59.63	720.50	460.56	530.63	28.17
SFCP	694.07	890.07	173.39	425.24	270.32	678.42	171.22
MZST	910.57	911.33	143.69	618.53	364.45	605.32	28.43
SFCPT	820.93	892.82	211.95	441.32	250.62	694.23	113.72

CON: Concentrate; LH: Lucerne hay; MS: Maize straw; SFCP: Sunflower-chickpea hay; MZST: CON+LH+MS; SFCPT: CON+LH+SF-CP; DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; IVDMD: *In vitro* dry matter digestibility; EE: Ether extract.

Animal variables

Milk yield in SFCPT with the sunflower-chickpea hay was significantly higher (P<0.001), at 10% more than the control diet (MZST), and there was a significant increase (P<0.05) in protein, total solids, and solids non-fat content over the conventional ration (MZST) (Table 3).

There were no significant (P>0.05) changes in body condition score, but the SFCPT treatment showed a significant (P<0.009) 1.02 kg higher live weight gain during the experiment than that for goats on the MZST treatment.

Total dry matter intake was 11.2% higher (P<0.001) in the conventional MZST ration than in the experimental SFCPT treatment, and refusals were also significantly (P<0.001) higher at 0.24 kg DM/goat/day in the SFCPT treatment compared to 0.12 kg DM/goat/day in MZST.

Table 3. Productive response of goats, milk composition, live weight, dry matter intake and forage refusals by treatment.

	MZST	**SFCPT**	**SEM**	**P-value**
Milk yield (kg/goat/day)	0.97	1.07	0.01	0.001
Milk fat (g/kg)	33.81	34.50	0.54	0.215
Milk protein (g/kg)	33.19	34.50	0.55	0.050
Total solids (g/kg)	121.14	122.96	1.02	0.017
Solids non-fat (g/kg)	89.81	94.00	0.90	0.001
Live weight change (kg)	2.64	3.66	0.26	0.009
Body condition score	6.27	6.44	0.23	0.152
TDMI (kg DM/goat/day)	1.07	0.95	0.06	0.001
Forage refusal (kg DM/goat/day)	0.12	0.24	0.12	0.001

MZST: concentrate + lucerne hay + maize straw; SFCPT: concentrate + lucerne hay + sunflower-chickpea hay; TDMI: total dry matter intake; SEM: standard error of the mean.

Discussion

Feeding dairy goats with hay obtained from sunflower intercropped with chickpea led to higher animal performance compared to the conventional diet based on maize straw, highlighting the prospects for improving the productivity of these systems as put forward by Rao *et al*. (2015) and Makkar (2016).

The decision to associate sunflower and chickpea stemmed from the benefits of legumes associated with other crops not only in terms of forage quality for animal feed, but in terms of agronomic, economic, and environmental benefits (Maxin *et al*. 2016) given by the nitrogen fixing of legumes.

However, no references were found on the use of this association as animal feed, so reference is done to literature on the use of sunflower or chickpea monocrops to contrast and discuss findings of this work.

Chemical composition of diets

Maize straw has been described as a roughage high in fiber, with low digestibility and poor protein content (Fuentes *et al*. 2001), as was observed in this experiment.

On the other hand, the sunflower-chickpea hay had better nutritive quality, with less NDF and ADF compared to maize straw, and a higher content of CP and EE resulting in 28% higher IVDMD (Table 2).

Ether extract and CP contents of the sunflower-chickpea hay were lower compared to other results that have evaluated sunflower as a forage source (Rodrigues-Gandra *et al*. 2017; Sainz-Ramírez *et al.* 2020), in spite of being harvested at similar maturity stages; but fiber contents were lower to values reported for sunflower silage by Guney *et al*. (2012) and Aragadvay-Yungán *et al*. (2015).

Sunflower as a forage source is reported to be high in fiber and low digestibility (Demirel *et al*. 2009) compared to other forages. Forages with high fiber content reduce the digestibility of the ration, the synthesis of rumen microbial protein and the supply of energy (Gottardo *et al*. 2017). The association of sunflower with chickpea, however, resulted in lower fiber content than sunflower in monoculture (Table 2), given that chickpea forage is low in fiber (Herrera-Flores *et al.* 2019). Nonetheless, in spite of its lower fiber content, the experimental SFCPT ration showed a lower dry matter intake, perhaps due to a higher concentration of lipids in the diet (28.4 g/kg DM in MZST vs. 113.7 g/kg DM in SFCPT).

Milk yield and composition

Results from work analyzed by Sanz-Sampelayo *et al*. (2007) suggested that increased lipids in the diet do not affect the net intake of energy or milk yields in goats, but positively affect, in most cases, the milk fat content, which was not observed in the current experiment. Energy foods in the diet increase the synthesis of microbial protein in the rumen, as well as the concentration of propionic acid, thus increasing milk production (Hills *et al*. 2015; Vicente *et al*. 2017). In spite of lower DM intakes, this effect was observed in the SFCPT ration, where sunflower seeds in the hay with a high lipid content (Rodrigues-Gandra *et al*, 2017), may have favored the increase in milk yields in the SFCPT experimental diet.

Contrary to reports by Chilliard *et al*. (2003), who did not observe differences in milk yield when supplementing the diet of milking goats with 3% fish oil in mid and late lactation, milk yield in the work here reported was higher in the SFCPT treatment.

Diet composition does influence the milk composition of dairy goats, due to factors such as energy intake (Sanz-Sampelayo *et al.* 2007; Sanz-Ceballos *et al.* 2009). Feeds with a high energy content increase the content of milk fat and protein content in milk (Kalač and Samkora, 2010), although only the increase in milk protein content was significant in the work found here, with no significant effect on milk fat content.

Milk fat is the component most susceptible to changes in the diet and of great importance in assessing the quality of milk. Kalač and Samkora (2010) and Gottardo *et al.* (2017) showed a positive correlation between protein content in the diet and fat content in milk, although in the work here described there were no significant differences between treatments for milk fat content even though SFCPT had over 47% higher CP content than MZST.

The effect of supplementary fats in the diet on the secretion of fat in milk has a minor effect in mid and late lactation compared to early lactation (Chilliard *et al*. 2003), due to the action of the anabolic enzymes on adipose tissue involved in *de novo* milk-fat synthesis, as well as to the lipase lipoproteins active after the peak of lactation (Soryal et al. 2004; Deshwala *et al.* 2020).

Results from the present experiment were similar, in terms of milk fat and protein, to reports by Zucali *et al*. (2007) supplementing dairy goats at the peak of lactation in Italy with sunflower seeds; whilst Arco-Pérez *et al*. (2017) reported higher contents of milk fat and protein in goats fresh after parturition when including sunflower oil in the diet.

It was also notable that goats on SFCPT showed a liveweight gain 38.6% higher than on MZST, although there was no change in body condition score.

In conclusion, results show that feeding dairy goats with the SFCPT ration based on sunflower-chickpea hay increases milk yield and live weight gain.

Declarations

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Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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Authors' contribution

Aurora Sainz-Ramírez conducted the research, laboratory analyses, writing - original draft. José Velarde-Guillén contributed to the methodology establishment, writing - review and editing. Julieta Gertrudis Estrada-Flores contributed to the methodology establishment, writing - review and editing. Felipe López Gonzalez contributed to the methodology establishment, statistical analyses, writing - review and editing. Carlos Manuel Arriaga-Jordán contributed to the conceptualization, resources, writing - review, editing and translation, supervision and funding acquisition.

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