



Effect of Phytogetic Feed Additives (Herb-All™ COOL) on Milk Production and Fertility of Dairy Cows in Hot Seasons

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ABSTRACT

In the last years several countries suffered from unusual heat waves in summer. High temperature and the related heat stress (HS) diminish the productivity and reproductivity of dairy cows. The aim of this study is to compare the productive and reproductive parameters in dairy cows (n = 250 Holstein cows) supplemented with a phytogetic feed additive (Herb-All™ COOL in a dose of 12 g/cow/day) with the non-supplemented group (control) (n = 250 Holstein cows) in hot seasons. In the present trial, phytogetic feed additive (PFA) supplementation restored the animals' normal physiological feeding and rumination patterns in Italy (the temperature humidity index "THI" ranged between 77 and 81). It enabled a significant increase in milk production ($P \leq 0.05$). This beneficial effect could even last two months after the supplementation was discontinued. In spite of the increase in milk volume, the concentration of the main milk components (milk fat and proteins) remained unchanged. The cows supplemented with PFA were healthier and expressed fewer postpartum complications, requiring fewer medications (-28.5%) and reducing the need to cull high-producing cows later (-40.3%) ($P \leq 0.05$). Furthermore, PFA enabled significant improvements in fertility parameters (the period to confirmed pregnancy was reduced by 21.3%, the success rate at first insemination was more than doubled (+106.9%), and the total success rate after insemination was improved by 41.4%, where more than 68% of the cows were successfully inseminated) ($P \leq 0.05$). In conclusion, PFA supplementation of dairy cows was capable of restoring both reproductive and lactational potential to thermoneutral levels.

Keywords: Dairy cows, Herb-All™ COOL, Heat stress, Italy, Phytogetic feed additive, Reproduction.

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INTRODUCTION

During the hot summer, dairy cows undergo several behavioral and metabolic adaptations to combat the negative impacts of hot ambient conditions by increasing heat loss and decreasing heat production (Herbut *et al.*, 2021). The economic impact of heat stress (HS) is underestimated as it is not limited to seasonal reduction in milk production but also affects fertility and disease susceptibility and increases the culling rate from the herd. It also affects the future productivity of newborn calves exposed to HS during their intrauterine phase, as approximately 60% of the body weight of calves is gained in the last two months of pregnancy (i.e., during the dry period); intrauterine exposure to HS during the dry period results in a huge effect on calf health (Halli *et al.*, 2021). The impaired

performance of the calves due to exposure to HS persists with the calves for a long time, as the intrauterine exposure to HS induces epigenetic modifications in the calf epigenome; therefore, their low productive potential will continue during their adulthood and can even extend for at least two generations (Laporta *et al.*, 2020; Ouellet *et al.*, 2020). In the US dairy sector, the total annual losses due to HS may reach 1.5 billion USD. It was estimated that a heat wave of only three days would cost the farmers about 337 USD. The cost can increase by up to 2500 USD if a cow dies (Vriezen *et al.*, 2021). In India, the losses in milk production due to HS are estimated to be 1.8 million tons of milk/year, or 2% of total milk production, and are expected to reach 50 million tons in 2050 (Tao and Dahl, 2013; Das *et al.*, 2016; Ouellet *et al.*, 2020).

Although the maintenance energy of dairy cows increases by 30% during HS, their feed intake clearly decreases (on average 1.4 kg/d/cow, 12.7%) to avoid heat production resulting in negative energy balance. Therefore, the cows tend to produce less milk (by one third at least and up to 40% of their potential) in order to slow down the metabolism and decrease their energy requirements (Wheellock *et al.*, 2010; Ouellet *et al.*, 2020). However, the drop in milk production has several reasons, for instance, as a consequence of subclinical mastitis and metabolic challenges upon exposure to HS as HS is usually accompanied by the release of many inflammatory mediators and reactive oxygen species (ROS) during the transition phase (Lykkesfeldt and Svendsen, 2007; Herbut *et al.*, 2021).

Moreover, during the dry period, HS decreases the proliferative potential of the glandular tissue and duct system of mammary glands and diminishes the readiness of immune cells to defend the body, which is reflected in the decline of milk yield and weakness of the immune response in the subsequent lactation, respectively (Tao and Dahl, 2013; Tao *et al.*, 2018; Ouellet *et al.*, 2020). Besides culling for reproductive failure, HS also increases the culling rate due to lameness and claw affections. This can be induced directly (as the cows remain standing for a longer time to enhance heat radiation) and indirectly (as a consequence of rumen acidosis, as HS enhances lactic acid-producing microflora that ferment soluble carbohydrates in the rumen, and due to the loss of salivary buffering by drooling). The acidosis is a predisposing factor for lameness (Shearer, 2005; Zhao *et al.*, 2019).

Exposure to HS leads also to disturbances of different reproductive parameters due to hormonal disturbances that suppress the ovulation process and mitigate the sexual behavior; the estrus signs become less prominent and last shorter, and finally, cases of anestrus and silent heat become obviously more frequent. All these factors result in a drop in conception and pregnancy rates during the summer months to only 10-20% and 62%, respectively. The HS leads, additionally, to the decrease in the fetus survival rate due to the damaging effect and reduction in protein synthesis in fetal cells enhanced by oxidative stress (Das *et al.*, 2016; Roth and Wolfenson, 2016; Ouellet *et al.*, 2020). Moreover, pregnant cows exhibit higher susceptibility to stillbirth and dystocia than normal (Halli *et al.*, 2020). It is worthy to notice that the reduction in conception rate and interval between calving can also be attributed to the effect of HS on male fertility (Halli *et al.*, 2020). A complete understanding of the mechanisms standing behind the physiological changes is essential to developing new approaches for reversing these negative effects of HS (Negrón-Pérez *et al.*, 2019). Phytogetics, such as herbs, which have

phytonutrients, phytochemicals, and essential oils, have been developed as alternative growth promoters to improve the quantity and quality of animal products (Wang *et al.*, 2024). Additionally, phytogetic feed additives (PFA) have been used to improve the reproductive performance of farm animals, due to their antioxidant and anti-inflammatory properties (Swelum *et al.*, 2021).

Terminalia bellirica (Gaertn.) Roxb. (Family Combretaceae), often known as '*Belleric myrobalan*,' is a large deciduous tree found in the Indian subcontinent, Nepal, Sri Lanka, and Southeast Asia (Akter *et al.*, 2019). Previous research suggests that *T. bellirica*'s compounds may contribute to its health advantages, including antipyretic, antioxidant, anti-inflammatory, and immunomodulatory properties (Al-Harrasi *et al.*, 2022). *Andrographis paniculata* is a popular herb in East and Southeast Asia, belonging to the Acanthaceae family, which is identified as the "King of Bitter" (Jiang *et al.*, 2021). *A. paniculata* has anti-inflammatory, antibacterial, antipyretic, and immunostimulant activities (Julaton *et al.*, 2022).

The diverse pharmacological effects of such PFA are exhibited by its various bioactive secondary metabolites, such as alkaloids, flavones, lignans, tannins, phenols, terpenoids, glycosides, and saponins (Akter *et al.*, 2019; Owoade *et al.*, 2021). Lee *et al.*, (2019) showed that dietary incorporation of a combined mixture of PFA has the potential to improve the milk yield and health status of Holstein cows under mild to moderate heat stress. Additionally, Abulaiti *et al.*, (2024) mentioned that the use of dietary PFA enhances the reproductive traits of Holstein dairy cows under summer conditions. Hamzah *et al.*, (2016) stated that supplementation of *Andrographis paniculata* to lactating goats increases the milk production due to their antioxidant effect.

Moreover, Ran *et al.*, (2022) observed an improvement in reproductive performance and the anti-oxidative capacity of periparturient dairy cows supplemented with *Andrographis paniculata*. Furthermore, Varshney *et al.*, (2012) illustrated that *Terminalia bellerica* treats the cases of bovine clinical/subclinical mastitis, so improve the milk production. No comprehensive research has been done on the potential interactions between the different combinations of *T. bellirica* and *A. paniculata* PFA in dairy cows exposed to HS environments. Therefore, in the present work, Holstein dairy cows were supplemented by PFA containing *T. bellirica* and *A. paniculata* combination (Herb-All™ COOL) and the productive and reproductive parameters was compared with the non-supplemented group (control) in hot seasons.

MATERIALS AND METHODS

Animal and feed composition

The trial was carried out on a dairy farm in Italy. The tested dairy herd was divided into two groups: one supplemented with PFA (Herb-All™ COOL) (Treated) (n=250 Holstein cows) and the second group received the feed without additional supplementation (Control) (n=250). The tested animals were divided into smaller groups according to the number of milking seasons (first milking season, second milking season, or their third season/above). The cows were supplemented with 12 g of the natural herbal product Herb-All™ COOL / cow / day in a premix, mixed directly into the total mix ration (TMR), which was composed of maize, grass silage, alfalfa hay, and concentrates (soybean meal and cereals), in addition to the mineral and vitamin premix. *Terminalia bellirica* and *Andrographis paniculata* are two of the pre-standardized, tested herbs in the polyherbal preparation “Herb-ALL™ COOL” (Life Circle Nutrition AG, Hämmerli 2d, 8855, Wangen SZ, Switzerland). The herbal composition (as provided by the manufacturer) is as follows: polyphenols: 3.32 g GAE/100 g (GAE: gallic acid equivalents), 25.7% (%W/W) water-soluble extract value, 13.5% crude fiber, 6.5% crude protein, 2.5% crude fat, 8.5% crude ash, 0.02% sodium, 0.2% lysine, and 0.1% methionine, 8.9% humidity.

Temperature-humidity index (THI) during the trial period

The trial was carried out for four months (from the beginning of June to the end of September). During the trial, the mean temperature-humidity index ($THI=0.8*T + RH*(T-14.4) + 46.4$) was calculated (Mader et al., (2006). A humidity and temperature measurement instrument (HOBO U23 Pro v2 Relative Temperature/Humidity Data Logger, Onset Computer Corp., Cape Cod, MA) was installed in a safe area

within each pen, near the bed or feed bunk. Temperature and humidity data were recorded daily. Software provided by the company (HOBOWare, Onset Computer Corp.) was used to read the recorded temperature and humidity data. After the end of the field trial, the team continued to collect the data for an additional two months (until the end of November) to evaluate the long-term efficiency of Herb-All™ COOL even after stopping its supplementation in the feed.

Measured parameters

Several behavioral, productive and reproductive parameters were monitored during the trial period. The parameters included: behavioral changes (signs of heat stress & time spent in feeding and rumination), productive parameters (milk volume, milk solids, number of culled cows & medical treatments per pregnant cow) and the reproductive parameters (period to first insemination, success rate of first Insemination (%), total success rate Insemination (%), period to confirmed pregnancy (d), gestation length (d) & dry period (d)).

Statistical analysis

Results were summarized in tables as means and standard errors (SE). Statistical inference was tested using independent sample t test. Significance was indicated at ($P \leq 0.05$). Data analysis was performed using PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The measured temperature, humidity and THI values are listed in **Table 1**. During the six months of monitoring (from May to November), the THI ranged from 62.0 to 80.6. However, during the four months of herb supplementation (June to September), the THI ranged from 71.4 to 80.6.

Table 1. The recorded temperature, humidity and THI values during the experimental period.

	May	Trial period				October	November
		June	July	August	September		
Temperature (°C)	18	23	27	29	26	24	17
Relative humidity (RH)	78	77	75	75	76	76	77
THI	63.6	71.4	77.5	80.6	76.0	72.9	62.0

Effect of PFA (Herb-All™COOL) on behavioral changes

Heat stress is usually bound to behavioral changes dealing with cow's feeding and rumination attitude. Supplementation with PFS (Herb-All™COOL) led to diminishing such behavioral changes accompanying HS. It could be easily observed that cows supplemented with the herbal preparation consumed more feed and spent more time in rumination.

Effect of PFA (Herb-All™COOL) on milk production

Milk production increased in average 3.5 kg / day / cow during the trial period (June – September) up on supplementation with PFA (Herb-All™COOL) ($P \leq 0.05$). There was an increase in milk volume which continued even after the stoppage of product supplementation for the following two months. Milk composition remained unchanged, where there were no significant differences between trial and control groups. The means were around 4.0% fat and 3.4% protein (Table 2, Fig. 1).

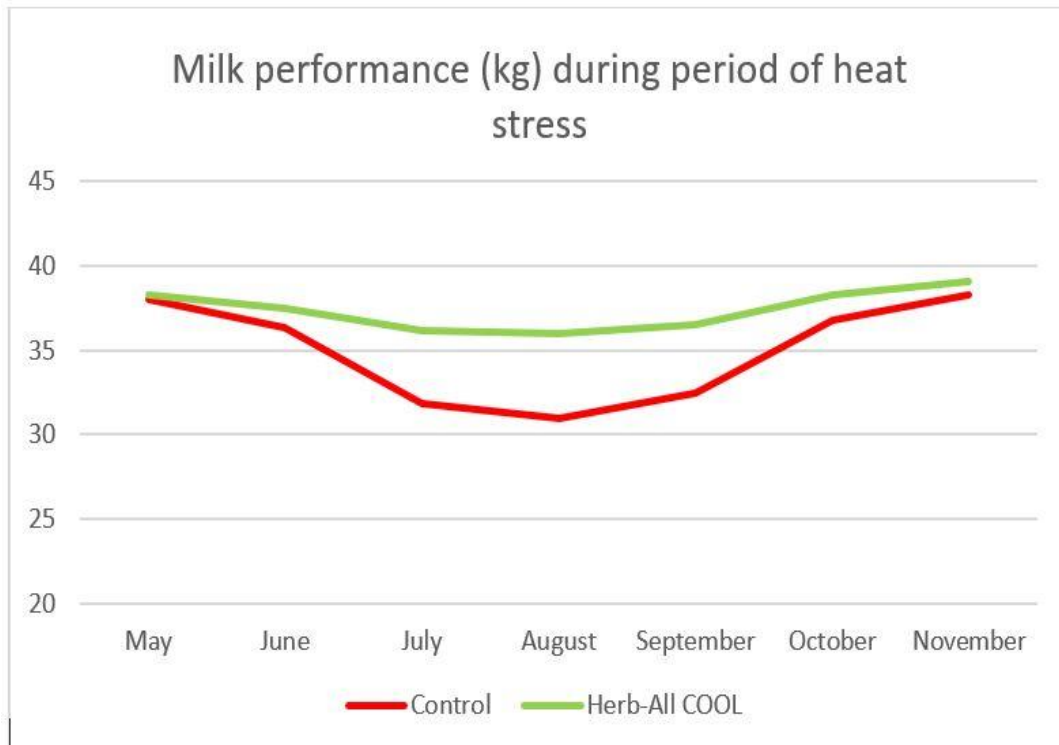


Fig.1: Volume of milk produced during the trial

Table 2. The reported quantities of milk production in both groups during the trial

	May	Trial period				Oct.	Nov.	Mean (June-sept.)
		June	July	August	Sept.			
Control group	38.00± 0.58	36.38± 0.66	31.86± 0.87 ^b	30.96± 0.78 ^b	32.49± 0.92 ^b	36.77± 0.81	38.32± 0.72	32.92± 0.69 ^b
Herb-All™ COOL	38.30± 0.66	37.50± 0.87	36.20± 0.72 ^a	36.00± 0.58 ^a	36.50± 0.72 ^a	38.30± 0.87	39.10± 0.58	36.55± 1.01 ^a
Difference	0.30	1.13	4.34	5.04	4.02	1.53	0.78	3.63

^{a,b} Means within a column with different superscripts significantly differ ($P \leq 0.05$).

Effect of PFA (Herb-All™COOL) on general health condition following calving

Supplementation with PFA (Herb-All™ COOL) reduced the occurrence of inflammatory diseases associated with calving significantly. The need of medical treatment was 1.5 treatments per cow compared with 2.1 in the control group (-28.6%) ($P \leq 0.05$). The number of somatic cells count in milk and incidence of mastitis were significantly reduced (data not available). As a result, the total number of the culled cows was reduced by 40.3% in the tested group ($P \leq 0.05$). (Table 3).

Table 3: The impact of supplementation with Herb All COOL on general health status

Group	Lactation season	Culled cows	medical treatments per pregnant cow
Control	1	4.00±0.15	1.80±0.58 ^a
Herb-All™ COOL		4.00±0.58	1.30±0.12 ^b
Diff. (%)		0.00%	-27.78%
Control	2	11.00±1.73	2.20±0.12 ^a
Herb-All™ COOL		9.00±0.58	1.70±0.06 ^b
Diff. (%)		-18.18%	-22.73%
Control	3 - >3	52.00±1.58 ^a	2.30±0.12 ^a
Herb-All™ COOL		27.00±1.73 ^b	1.40±0.17 ^b
Diff. (%)		-48.08%	-39.13%
Control	Total	67.00±1.15 ^a	2.10±0.58 ^a
Herb-All™ COOL		40.00±1.73 ^b	1.50±0.12 ^b
Diff. (%)		-40.30%	-28.57%

Effect of PFA (Herb-All™COOL) on fertility parameters

The supplementation of the dairy herd with PFA (Herb-All™COOL) had significant ($P \leq 0.05$) positive effects on the investigated fertility parameters of the herd including the success rate at first insemination was more than doubled of the control group (+106.9%), and the total success rate after insemination was improved by +41.4%. Meanwhile, the period to confirmed pregnancy was reduced by 21.3%. Moreover, the number of calving was also improved by 4.8% (Table 4 and Fig.2).

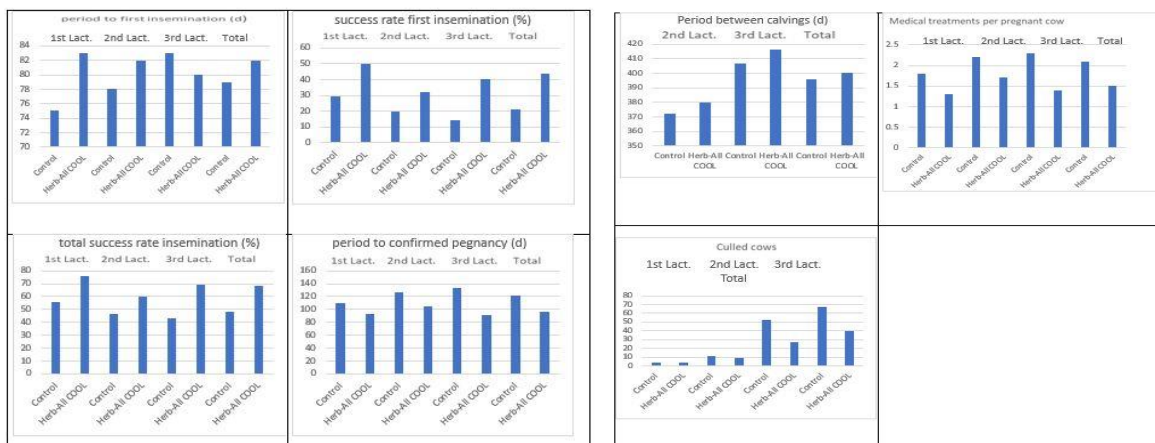


Fig. 2: The diagrams compare the delivered data from both groups involved in the present work. Different productive and reproductive parameters are presented including the No. lactation season, for the period to first insemination (d), the success rate first insemination (%), The total success rate insemination (%), The period to confirmed pregnancy (d), The period between calving (d). The Medical treatment per pregnant cow, and the culled cows.

Table 4: The effect of PFA (Herb-All™COOL) supplementation on the reproductive parameters.

Group	Lact-ation season	period to first insemination (d)	success rate first Insemination (%)	total success rate Insemination (%)	period to confirmed pregnancy (d)	period between calvings (d)	gestation length (d)	dry period (d)
Control	1	75.00± 2.89 ^b	29.41± 1.15 ^b	55.50± 5.20 ^b	110.00± 2.58 ^a		275.00± 4.62	
Herb-All™ COOL		83.00± 1.73 ^a	50.00± 2.31 ^a	76.00± 3.46 ^a	93.00± 3.18 ^b		277.00± 2.31	
Diff. (%)		10.67%	70.01%	36.94%	-15.45%		0.73%	
Control	2	78.00± 2.31	19.51± 0.58 ^b	46.30± 2.89 ^b	127.00± 4.62 ^b	372.00± 1.44 ^b	275.00± 4.33	59.00± 2.89
Herb-All™ COOL		82.00± 1.15	32.00± 1.73 ^a	60.00± 2.02 ^a	104.00± 4.04 ^a	380.00± 2.02 ^a	278.00± 3.18	63.00± 1.44
Diff. (%)		5.13%	64.02%	29.59%	-18.11%	2.15%	1.09%	6.78%
Control	>3	83.00± 1.73	14.29± 1.87 ^b	42.90± 2.08 ^a	134.00± 4.33 ^a	407.00± 3.46 ^b	280.00± 3.46	69.00± 1.73
Herb-All™ COOL		80.00± 1.15	40.48± 2.02 ^a	69.00± 2.60 ^a	92.00± 3.18 ^b	416.00± 1.73 ^a	276.00± 2.31	68.00± 1.15
Diff. (%)		-3.61%	183.28%	60.84%	-31.34%	2.21%	-1.43%	-1.45%
Control	Total	79.00± 1.73	21.10± 1.15 ^b	48.30± 4.62 ^b	122.00± 5.77 ^a	396.00± 3.46	277.00± 2.89	66.00± 4.62
Herb-All™ COOL		82.00± 2.31	43.66± 3.46 ^a	68.30± 2.89 ^a	96.00± 4.04 ^b	400.00± 5.20	277.00± 6.35	66.00± 2.30
Diff. (%)		3.80%	106.92%	41.41%	-21.31%	1.01%	0.00%	0.00%

^{a,b} Means within a column with different superscripts significantly differ ($P \leq 0.05$).

DISCUSSION

The negative impact of HS on the production levels of dairy animals is well documented. This is attributed to physiological, hormonal, behavioral, pathological and metabolic changes in response to the HS in order to increase heat loss and to avoid the production of extra heat. It was estimated that milk production decreases by 0.4 kg/cow/day when the THI increases only one unit above 69. The half of the reduced milk volume can be attributed to the reduction in feed intake, the second half results from the negative effects of HS on the metabolic process and hormonal disturbances in response to heat, which is equivalent to 3.6 kg/d/cow (10.3%) on average during the hot season (Tao and Dahl, 2013; Das et al., 2016; Ouellet et al., 2020).

In the present work, the THI value was 71.4 in the first trial month, during which the effect of HS on milk production was limited. In the next months, with the increase in THI value, milk reduction in the control group continued and peaked in August (THI value was 80.6). At the end of the monitoring period (November), the THI sank again below the threshold level so that there was no negative impact of HS on milk production. Although the supplementation with PFA (Herb-All™ COOL) ended in September (THI 76), the positive effect on milk production extended to cover October (THI 72.9) before sinking gradually. It can be imaginable that the effect of supplementation with PFA (Herb-All™ COOL) on milk production during summer

months might last for a longer time (increased numerically) after its removal from the feed formulation if the ambient temperature and humidity continued to exceed the HS-inducing level in autumn.

Upon supplementation with PFA (Herb-All™ COOL), the most obvious difference between the two groups in the volume of produced milk was seen in August (5.04 kg/cow/day; THI: 80.6), while the lowest was recorded in November (0.782 kg/cow/day; THI: 62), which indicates the potential of PFA (Herb-All™ COOL) to relieve the negative effects of HS on milk production. This effect can persist for at least two months after the drop of the herb from the formulation. The increase in milk production is also attributed to the reduction in the number of mastitis cases in the supplemented group. Ranjani et al., (2022) demonstrated that *T. belerica* has antioxidant and antimicrobial activities and could be utilized as an alternative to antibiotics to treat the bovine mastitis to improve the milk production. In addition, Hossain et al., (2021) reported *A. paniculata*'s efficacy against *Staphylococcus aureus* and *Escherichia coli* (the causative agents of subclinical and clinical mastitis). *T. Bellirica* and *A. paniculata* contain numerous bioactive metabolites, including alkaloids, flavonoids, tannins, and phenols (Akter et al., 2019; Owoadé et al., 2021), that have anti-inflammatory, antibacterial, and antipyretic effects (Julaton et al., 2022; Sharma et al., 2021). The correction of the milk curve during unfavorable hot seasons via the supplementation with

PFA (Herb-All™ COOL) provides flexibility to extend the calving season over the whole year. Previously, it was advisable to adjust the calving season and high milk production curve in moderate climatic months to avoid the deviations in milk curves due to high THI values (Zamorano-Algandar *et al.*, 2022).

Published data reported that about one-third of dairy cows develop at least one clinical health disorder (mastitis/metritis, digestive/respiratory problems, or lameness) during the first 21 days after calving. Such postpartum clinical diseases that occur during the first 3 weeks in the milking season, even if treated, express persistent long-term negative effects on cow productivity (lactation performance) and fertility and consequent culling (Carvalho *et al.*, 2019; Ehsanollah *et al.*, 2021). However, the data delivered from the present work showed that PFA (Herb-All™ COOL) supplementation leads to the reduction of post-calving inflammatory disorders of the genital tract and udder and disorders associated with conception failure that require medical interference, which avoids premature culling of valuable animals from the herd by 40%.

Besides the milk volume and compositions, additional reproductive parameters are also negatively influenced by HS, including the length of lactation & dry period, interval between calving, period to confirmed pregnancy, success rate at first insemination, and the total success rate after insemination (Putney *et al.*, 1989; Rensis and Scaramuzzi, 2003; Sheikh *et al.*, 2017; Dahl *et al.*, 2019; Toledo *et al.*, 2022). Gernand *et al.*, (2019) showed that HS, especially in early lactation, had a negative impact on cow productivity and female fertility. Moreover, Toledo *et al.*, (2022) demonstrated that exposure of dairy cows to HS has detrimental effects on reproductive performance and reduces the immune status and health. There are currently several tools available to alleviate these negative effects on cow reproduction, such as supplementing with certain herbal feed additives (particularly antioxidants).

Several commercial herbal feed additives can only mitigate the effects of HS on fertility; however, some of them may lead to long-term reproductive disorders due to their content of estrogen-like substances, which disturb the reproductive cycle of the cows (Swelum *et al.*, 2021). Ran *et al.*, (2022) showed that *A. paniculata* improved the health status and reproductive performance of dairy cows by enhancing immunity, antioxidant function, and improving energy metabolism and inflammatory response, therefore, improving the welfare and well-being of them. Additionally, Fayed *et al.* (2024) observed that PFA (Herb-All™ COOL) effectively mitigated heat stress's negative impacts due to its antipyretic, antioxidant, and immunomodulatory activity. The delivered data from

the present work revealed that supplementation of dairy cows with PFA (Herb-All™COOL) corrects the physiological disorders resulting from the exposure to high surrounding temperature. By so doing, according to the obtained results, PFA (Herb-All™COOL) leads to the neutralization of the negative impact of HS on both productivity and fertility, which reflects positively on the farm's profitability, animal general health condition, and animal welfare.

CONCLUSION

In the present work, PFA (Herb-All™COOL) supplementation containing a *Terminalia bellirica* and *Andrographis paniculata* combination restored normal rumination and feeding patterns, resumed milk production winter curve, and reduced post-parturient inflammatory and reproductive disorders under a 77 to 81 Temperature-Humidity Index (THI), therefore reducing the medication needed. Consequently, the need to cull cows in production was reduced by (-40.3%). Other parameters, including the period to confirmed pregnancy, success rate at first insemination, and total success rate after insemination, were also clearly improved. The increase in milk production curve could even persist for an additional two months after stopping herb supplementation.

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Statement of Animal Rights

The ethical regulations governing animal experiments were strictly followed.

Conflict of Interest

The authors declare no conflict of interests.

Data availability

All data delivered or analysed during the investigation are included in the present work.

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