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Curcumin Enema might Regulate Intestinal Barrier Functions and Calf Hygiene Scoring

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THE goal of the current study was to evaluate the effectiveness of a 10-day rectal enema curcumin treatment for calves suffering from diarrhea and its effects on intestinal barrier functioning and calf hygiene scoring. **Material and Methods**: Thirteen diarrheic calves in all were enrolled, and the effectiveness of the rectal curcumin treatment was assessed by evaluating calf hygiene and health scoring. **Results:** Fecal scores ranged between1-3 (7 calves had scores of 3) prior curcumin treatment (days 0), whereas after treatment (day 10), these ranged between 0 to 1. (10 calves with score 0) ($p<0.05$). However, calf hygiene scores increased from 4 to 9 (7 of them above 7) before treatment (day 0) to 1-3 (scoring 1 for 7 calves) with a statistically significant change ($p<0.01$) after treatment (day 10). This study supports that rectal curcumin may have assisted in reducing dirtiness and improving hygiene conditions, as measured by scoring for the interpretation as to whether calves had a comfortable, clean, and dry area to lie down.

Keywords: Calves, Curcuma longa, Health status, Hygiene condition, Rectal enema.

Introduction

Management of dairy animals is arousing great interest to agriculture engineering, veterinary surgeons, farmers, and consumers also. Veterinary surgeons and agricultural engineers, in their related area, in an attempt to modify animal welfare for prevention of disorders, while endeavoring to stabilize economical, ethical, and food safety regulations [1]. Given this valuable data, cleanliness and hygiene were denoted as significant factors for achievement of those purposes. Several various methodology were taken into consideration form determining the efficacy hygiene status among calves' health [2-9]. To those of, also in our interest, Panivivat et al. [2] validated a scoring system targeted as tail head region, thighs, and legs were determined even if soiled with fecal material. The latter validation was performed by Sutherland et al. [3-5] and Graham et al. [9]. Lundborg et al. [10] briefly performed another scoring for 8 different body parts of calves. In the present study we preferred another scoring system adopted by Kellermann et al. [1], in which denoted as calf hygiene scoring.

Curcumin, a natural compound with anti-inflammatory, antioxidant, and anti-neoplastic properties, has been used for a long time. However, its poor bioavailability when taken orally, resulting in minimal absorption and low levels in the bloodstream, has raised doubts about its potential as a therapeutic agent [11]. A new approach suggests using the gut as the target site for curcumin administration, bypassing the issue of poor oral bioavailability. This approach explores the role of gut barrier function in

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curcumin's effectiveness against various diseases. Curcumin is taken up by intestinal epithelial cells through an unknown mechanism [12]. Once inside these cells, curcumin activates or modifies several signaling pathways that help protect the intestinal barrier from disruptions caused by external dietary factors or internal events like ischemia/ reperfusion injury. This leads to a reduction in luminal bacteria and bacterial products (endotoxemia), resulting in the observed benefits of curcumin. By alleviating chronic inflammation at the intestinal level, curcumin shows promise [13]. Studies have demonstrated the effectiveness of curcumin against parasites in calves when administered orally [14] or rectally [15].

Zonulin, in general, solely physiological influence of intercellular tight junctions determined to a degree that is implied in trafficking of macromolecules and, thus, in tolerance/immune response balance [16]. Zonulin has been the subject of diarrhea [17], heat stress [18], heat stress and intestinal barrier disruption [19] among calves. Given the definition of leaky gut among calves [19-21], further validation of selected biomarker such as zonulin should be analyzed in depth among calves. Hence the purpose of the present study was therefore to investigate the efficacy of 10 days duration rectal enema curcumin treatment to those of calves with diarrhea and its consequences on intestinal barrier functions and calf hygiene scoring.

Material and Methods

Animal study

The present study was performed in two different diary industry framing units located at the same geographical location in Aydin Province [Latitude: 37° 50' 16.8576'' N, Longitude: 27° 50' 44.016'' E]. Both farms were close to the city center at Aydin Municipality. The present study was financially supported by Aydin Adnan Menderes University Research Funding Unit (ADU BAP) with project no: SUMYO-23001.

Calves and housing facilities

Similar to previous methodology as described [18] the present research was conducted on 13 calves (4 males/9 females) aged 7 to 13 weeks, on two closely related private commercial farms in Aydin Municipality, Turkey, as aforementioned above. Self-control group was enrolled, as each calf enrolled as its control comparatively (meant prior to and thereafter curcumin enema), denoting the previous analyte day [18]. Entire calves were housing individually.

Health status assessment

Health scoring was adopted from what have been previously described [21] along with evidenced based veterinary literature as shown in Table 1.

Interpretation demographics

Fecal scores ≥2 denoted as diarrhea [12]. A skin time return greater than 3 s were described as dehydration and even if greater than 5 s, as severe dehydration [13]. A calf was considered to have a fever if the rectal temperature was ≥39.5°C.

Calf hygiene scoring

As was described previously [1] this scoring system evolved, three risk zones [belly, the side, and the rear] were suggested/denoted, and each risk zone has been addicted with an individual score. The summation of the risk zone resulted within the calf hygiene scoring. Briefly responsible people, calculating the calf hygiene scoring, were located either on the side of each calf at 90° angle and through the middle of the longitudinal side of the animal, from 0.5 to 2 m step forwards (for inspection of the belly and the side), or 0.5 to 2 m and in a squatting position (similarly), on behind the animal through middle of its longitudinal axis [1]. Interpretation of the numeric proportion of dirtiness of the anatomical locations belonging to risk zones were denoted as a score from 1 to 3 for each individual, as shown in Table 2.

Intestinal integrity: Zonulin

To determining zonulin levels before and after rectal enema curcumin administration, blood sample was collected in serum tube and serum was separated and frozen until analysis. Bovine specific zonulin was conducted with ELISA (MyBiosource ELISA kits, USA) in accordance with the manufacturer's instructions.

Curcumin enema treatment modality

Previous description [15] was adopted and foley catheter was manually forwarded rectally in each session to all calves enrolled. With the guidance of another researcher involved at this study, performer veterinary surgeon moved 15 cm from rectum, at least, and administered 97% pure curcumin compound in 15 ml olive oil.

Statistical analyses

The descriptive statistics of the data obtained through analyses and observations were calculated, and the results obtained through scoring were presented as median, while zonulin levels were presented as mean and standard error. Comparisons between pre- and post-treatments

were performed using the Mann-Whitney U test, and analysis results with a p-value less than 0.05 were considered statistically significant. Graphpad Prism Ver. 9.5.1 software was utilized for all analyses.

Results

Demographic data

In a total of 13 Holstein and Jersey calves were enrolled at the present study for interpretation of calf hygiene scoring, none of the calves were excluded and all calves completed the research. All calves were suckling beginning with day 0 to approximately twelve weeks housed in individual pens. Interpretation took place on 10 days in relationship with rectal enema administration. Each calf was rated by each 5 researchers individually for observer reliability. Furthermore, photographic records (Figs. 1-4) of all calves were taken for each 3-risk zone. All pictures (Figs. 1-4) were then analyzed for calculation of the actual proportions of soiled locations participated within the photographed risk zones of the calves [1].

Scoring data

To those of solely fecal scores ≥2 denoted as diarrhea [22] were enrolled in all involved calves. All calves with a skin time return greater than 3s [dehydration], were enrolled whereas due to not risk holding none of the calves with greater than 5s, were evolved [23]. None of the calves were classified as etiological interpretation nor responsible agents were detected. None of the calves had a fever.

Prior to curcumin treatment (days 0) faecal scores were deemed ranging between 1-3 (7 calves with scores of 3) whereas after treatment (day 10) ranged between 0 to 1 (10 calves with score 0) as shown in Fig 2. There was a statistical significance ($p<0.05$). On the other hand Calf hygiene scores before treatment (day 0) ranged between 4 to 9 (7 of them above 7) whilst switched to 1-3 (score1 for 7 calves) with a statistical significance ($p<0.01$) after treatment (day 10) (Fig. 5).

Zonulin leves

Prior to curcumin enema zonulin levels (ng/mL) changed between 29,4 to 80,4 whereas following curcumin enema min-max. Values were 16,9 to 42,6. Statistically significant levels of zonulin levels were obtained following curcumin enema ($p<0.01$) (Fig. 6).

Discussion

Prior to curcumin enema zonulin levels (ng/mL) changed between 29.4 to 80.4 whereas following curcumin enema min-max. Values were 16.9 to 42.6. Statistically significant levels of zonulin levels were obtained following curcumin enema ($p<0.01$) (Fig. 6).

Curcumin has long been denoted as a natural anti-inflammatory, antioxidant and anti-inflammatory ayurverdic. Given its poor bioavailability (for oral usage), there exists skepticism through its effective role [24] and to the present authors' knowledge several veterinary surgeons were unaware of its mechanism of action. Due to its trifling amount in circulation and markedly deduced levels at target cells, significant investigations are being performed directed towards elevated absorption [24].

Someone might speculate how curcumin can regulate intestinal barrier functions, as was the topic and purpose of the present study. This issue needs to be addressed for better understanding this manuscript. Thereafter the authors should in depth discuss what have been available in calves with curcumin as the subject of possible literature. As defined by "unconventional" mechanisms: i) adequate curcumin levels persisted in colonic mucosa, intestine is probably a significant location of its action [24], ii) the participation of the methoxy groups for anti-inflammatory respond belonging to curcumin provided evidence of proof for beneficiary efficacy of curcumin in the gut in colitis model [25], iii) due to its first pass metabolism and well recognized poor absorption, enterocytes are probably the targeted location for curcumin action (in the present study curcumin was given as rectal enema, which could be an advantage) [26], iv)curcuminoids are thoroughly subjected to metabolization via gut bacteria for production of metabolites presenting efficacy [27], v) curcumin has been well recognized to battle chronic inflammation and intestinal barrier dysfunction (which was also subject of this study) [24].

Zonulin pathway plays a pivotal role in intestinal barrier dysfunction and processing of disorders. One such pathway that is relevant to intestinal barrier dysfunction and the disease process is the zonulin pathway. Equivalent to bacterial toxin, namely zonula occludens, improper of increased zonulin production from the intestinal epithelial cells through the lumen

elevates paracellular permeability [16, 28]. Fecal concentrations of zonulin are detected as a clinical biomarker of intestinal permeability. Hence, interference that influence expression and/or arrangement of the latter tight junction proteins are probably modulating paracellular transport of luminal bacteria (its products) into the systemic circulation [24]. Unusual intestinal permeability likewise participated within the pathology of various autoimmune diseases [16]. Through detection of markedly increased concentrations of circulating gut bacterial products in idiopathic arthritis, Fotis et al. [29] claimed that the intestine is the origin of immune stimulation in arthritis. In agreement Ciccia et al [30] suggested that zonulin expression was elevated, in association with damaged intestinal mucosal barrier among ankylosing spondylitis cases. In addition, the latter researchers also claimed that modulation of monocyte activation via the latter bacterial products and zonulin could all participate as underlying pathogenesis. At the present study day 0 levels (ng/mL) were detected as 29,4 to 80,4 whilst after curcumin enema switched to 16,9 to 42,6 ($p<0.01$) (Fig. 6). These preliminary results were deemed available, presented that curcumin enema might be capable of influencing intestinal barrier functions.

In a prior study the efficacy of citrus seed extract, via rectal route, on hide cleanliness and stool consistency scores in diarrhoeic and non-diarrhoeic calves were investigated. Stool consistency and hide cleanliness were both scored on a scale of 0 to 3 in totally 18 calves [11 diarrheic and other relevant 7 healthy], citrus seed extract was administered (20 ml) to each group by rectal enema with (20 ml) for 1 week which resulted with declined scores (between 0-1) [17]. In the present study curcumin treatment (days 0) faecal scores were presenting values 1 to 3 (7 calves with scores=3) whereas day 10 values switched between 0 to 1. Interestingly 10 calves showed score 0 ($p<0.05$) (Fig. 2). Furthermore, calf hygiene scores on day 0 altered 4-9 (7 of them above 7) whilst changed to 1-3. Surprisingly score1 was evident for 7 calves ($p<0.01$) at day 10) (Fig. 5). All aforementioned data support preliminary findings that rectal curcumin might have helped removing dirtiness and correcting hygiene conditions as tabulated by scoring for interpretation of if calves have a comfortable, clean, and dry space to lie down.

Conclusion

It should n to be unwise to draw preliminary conclusion that curcumin enema, at recommended dosages in the present study might be capable of modulating intestinal barrier functions as evidenced by altered zonulin levels. Furthermore, declined calves' hygiene scores and relevant fecal scores could be dedicated to curcumin enema hygiene efficacy. Further studies are warranted at different dose regimes.

Conflict of interest

There are no competing interests.

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TABLE 1. Health assessment of present calves enrolled herein.

Health scoring					References
	Fecal scores				[17]
	0	1	2	3	
	Solid	Soft	Pasty/runny	Runny/ watery	ILIT
	Dehydration				[18]
	skin time return ≥ 3 seconds		≥ 5 seconds		

TABLE 2. Calf hygiene scoring chart.

	Calf hygiene scoring*
Score 1	0 to 10% of the area (no or little soiling)
score 2	over 10 to 30% (medium soiling)
Score 3	soiling of more than 30% of the area (heavy soiling).

* All types of soiling were taken into consideration with exclusion criteria [1]. The scores of the individual regions were summed up to yield the CHS ranging from 3 to 9.

Fig. 1. Individual calf enrolled at the present study with a calf hygiene score of 8, prior to treatment

Fig. 2. Individual calf enrolled at the present study with a calf hygiene score of 3, prior to treatment.

Fig. 3. Thereafter rectal enema curcumin treatment a calf with a hygiene score of 1

Fig. 4. Thereafter rectal enema curcumin treatment a calf with a hygiene score of 1

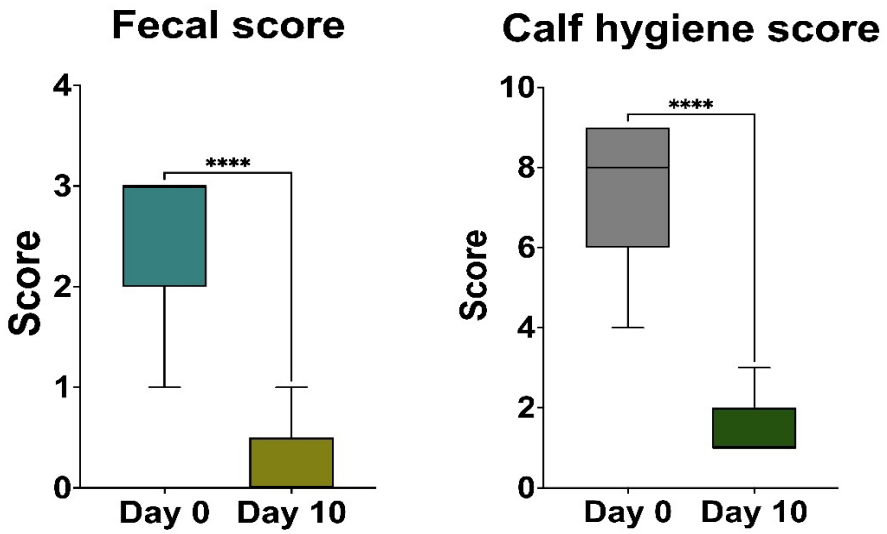

Fig. 5. Median Fecal score and Calf hygiene scores among calves enrolled at this study prior to and thereafter curcumin treatment ** (p<0.01)**

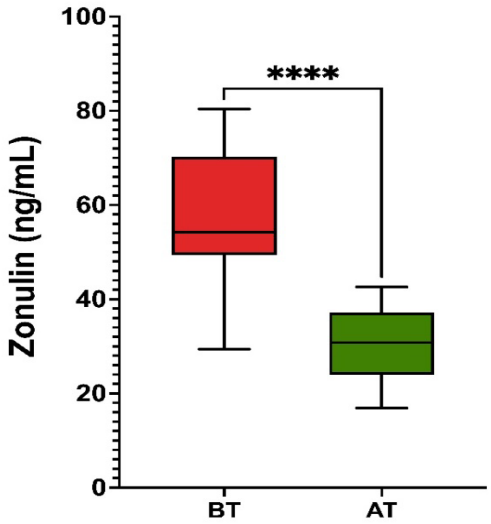

Fig. 6. Zonulin levels prior to and thereafter curcumin enema treatment** (p<0.01)**.

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