RESEARCH ARTICLE

Clinical Efficacy of Antimicrobial Agents in Combination with Flunixin Meglumine and Phenylbutazone on Acute Phase Response in Respiratory Disease of Calves

Wael M. El-Deeb[1,2]*, Mohamed A Rizk[2], Mahmoud M. Fayez[3], Hermine V Mkrtchyan[4] and Mahmoud Kandeel[5]

[1]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia; [2]Department of Veterinary Medicine, Infectious Disease, and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt; [3]Ministry of Agriculture, Al Ahsa Veterinary Diagnostic Laboratory, Saudi Arabia; [4]School of Biomedical Sciences, University of West London, St Mary's Rd, London, UK, W5 5RF; [5]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
*Corresponding author: weldeeb@kfu.edu.sa

A B S T R A C T

The aim of this study was to correlate the serum levels of acute phase proteins (APPs) and cytokines in response to treatment by various antimicrobial agents in feedlots calves (FL) naturally infected with *Mannheimia haemolytica (M. haemolytica)* and *Histophilus somni (H. somni)*. 840 feedlot calves in one farm in Al-Kharg region, Saudi Arabia were clinically examined for the presence of respiratory disease manifestations. The infection was confirmed using nasopharyngeal swabs. Blood samples from diseased animals were collected before and after (7 days) treatment for biochemical analysis of serum amyloid A (SAA), haptoglobin (HP) and cytokines tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-1 β (IL-1β), and interleukin-8 (IL-8). FL that were positive for *M. haemolytica* and/or *H. somni* (n=69) received treatment with one antibiotic, including tulathromycin (Tula; n=26 cases), florfenicol (FFC; n=19), tilmicosin (Tm; n=13), or ceftiofur (CEF; n=11) and one non-steroidal anti-inflammatory drug (Flunixin meglumine (FM; n=43) or phenylbutazone (PBZ; n= 26). We demonstrated the selective potent inhibitory effect of the administrated anti-inflammatory agents either FM or PBZ on the production of APPs and pro-inflammatory cytokines in FL infected with bovine respiratory disease (BRD). Our findings showed the antibacterial efficacy of FFC and Tm for the treatment of infected FL when administrated with either FM or PBZ. However, Tula was preferable to administrate in combination with FM for the treatment of FL with respiratory manifestations. Importantly, monitoring the sera level of Hp, IL-1β, and interleukin-8 (IL-8) in feedlots treated with either FM combined with Tula, FFC, or Tm or PBZ combined with FFC, and Tm has been effective in predicting the disease prognosis.

To Cite This Article: El-Deeb WM, Rizk MA, Fayez MM, Mkrtchyan HV and Kandeel M, 2021. Clinical efficacy of antimicrobial agents in combination with flunixin meglumine and phenylbutazone on acute phase response in respiratory disease of calves. Pak Vet J, 41(1): 71-77. http://dx.doi.org/10.29261/pakvetj/2020.078

INTRODUCTION

Bovine respiratory disease (BRD) is considered a major health issue and an uninterrupted challenge worldwide causing considerable economic losses among dairy and feedlot calves (FL) (Woolums *et al.*, 2018). The disease is manifested by lethargy, coughing, nasal discharge, dyspnea, tachypnea, septicemia, fever, hypoxia, anorexia and acute phase response (El-Deeb *et al.*, 2020).

The acute phase proteins (APPs) that synthesize in hepatic cells are released into blood circulation in the process of acute phase response (APR) against inflammatory conditions or infections (El-Deeb and Iacob, 2012; El-Deeb *et al.*, 2014; El-Deeb and Buczinski, 2015; El-Deeb and Tharwat 2015; El-Deeb and El-Moslemany, 2016a, 2016b; El-Deeb *et al.*, 2017). The detection of APPs and pro-inflammatory cytokine levels in the blood of diseased calves may possibly be used as a tool for BRD diagnosis and for evaluation of treatment responses (El-Deeb *et al.*, 2020).

Indeed, the early stage of BRD is characterized by a significant inflammatory response in the host, a condition that requires an urgent need for administration of nonsteroidal anti-inflammatory drugs (NSAIDs) (Lekeux, 1996). Subsequently, a combination therapy consisting of anti-inflammatory drugs and antimicrobials have become a preferable field protocol for respiratory disease treatment in cattle (Van de Weerdt and Lekeux, 1997; Rizk *et al.*, 2017). However, there is a paucity on the influence of anti-inflammatory drugs when administered in combination with other antimicrobial agents to calves either experimentally- or clinically diseased with *M. haemolytica* and *H. somini*. In this regard, the efficacy of several antimicrobial agents including enrofloxacin, danofloxacin, oxytetracycline, ceftiofur, amoxicillin/ clavulanic acid, tylosin, tilmicosin, sulfadimethoxine, florfenicol and tulathromycin have been reported for treatment of calves suffering from respiratory diseases (Benchaoui *et al.*, 2004; Rizk *et al.*, 2017).

The usage of APPs and pro-inflammatory cytokines sera levels for diagnosis of BRD or for monitoring treatment efficacy in pneumonic calves has previously been reported by other authors (Bednarek *et al.*, 2003; Gruys *et al.*, 2005; Rizk *et al.*, 2017; Joshi *et al.*, 2018). However, these studies had limitations of administrating only one or two antimicrobial agents. In addition, the identification and isolation of causative agents were not well categorized in these studies. Notably, no study correlated yet the serum levels of APPs and cytokines in response to treatment by different antimicrobial agents in calves clinically infected with *M. haemolytica* and *H. somini.* The results obtained from this study will aid to better understand the complex mechanism of APPs and cytokines productions in FL with RD under the field conditions. In this study, we compared the therapeutic effect of various antimicrobials (Tulathromycin (Tula), florfenicol (FFC), tilmicosin (Tm), ceftiofur (CEF)) administered in combination with NSADs (flunixin meglumine (FM) and phenylbutazone (PBZ)) on the levels of selected cytokines (IL-1 β, IL-8, interferon gamma (IFNγ) and TNF-α) and major APPs (Hp, and SAA) in FL infected with *M. haemolytica* and *H. somini*.

MATERIALS AND METHODS

Study design: 840 feedlot calves (4-12 months old) were examined in one farm in Al-Kharg region, Saudi Arabia between July and August 2016 in response to the complaint of a farm owner for the presence of respiratory disease manifestations and consequently deaths among the farm animals. The farm records revealed that all animals were previously vaccinated against viruses causing bovine BRD.

All animals were clinically examined for signs of BRD (fever, nasal discharge, lacrimation, wheezes, crackles, congested mucous membrane, depression, decreased appetite, and difficult respiration). Nasopharyngeal swabs (Culture Swab-Kalayjian, Patterson Veterinary Supply Inc., USA) and blood samples (5ml) were collected from each animal with fever and with one or more other RD signs (n=250) for screening.

Diseased calves that tested positive for *H. somni* and/or *M. haemolytica* (n=69) received treatment with one

antibiotic (Tula "Draxxin®", n=26 cases, FFC "Nuflor®", n=19, Tm "Pulmotil®", n=13, or CEF "Excenel®", n=11) and one anti-inflammatory drug (FM, n=43 or FBZ, n= 26). Calves that did not response to the treatment (n=20) were necropsied and pharyngeal swabs, tracheobronchial lavage, heart-blood and lung samples were collected for further bacteriological and virology examinations. The treatment regime and doses for each antibiotic and anti-inflammatory drug were designated according to the manufacture's recommendations.

Blood samples were taken from diseased cases before and after (7 days) treatment. Recovered serum samples were stored in Eppendorf tubes at -80°C for further detection of biochemical variables including serum amyloid A (SAA), haptoglobin (HP) and cytokines (TNF-α, IFN-γ, IL-8, IL-1β,).

Isolation and identification: The external surface of lung specimens was seared by a hot spatula. Following this, a parallel incision was made, and the deep tissues were swabbed.

Nasopharyngeal swabs and lung specimens were inoculated on sheep blood agar containing 0.5% yeast extract (Oxoid Ltd, Basingstoke, UK) and 5 mg/mL of bacitracin and Colombia blood agar (Oxoid Limited, UK) supplemented with 5% sheep blood and selective antibiotics as described previously (Slee and Stephens, 1985). Inoculated plates were incubated in microaerophilic atmosphere containing 5% CO_2 for 48 h at 37°C. Presumptive *M. haemolytica* and *H. somini* were further identified using the VITEK 2 Compact (BioMérieux, France).

Nucleic Acid extraction: RNA and DNA extractions (nasopharyngeal swabs, lung tissues and bacterial isolates) were prepared as described previously (Klima *et al.*, 2014), using RNeasy Mini Kit and QIAamp DNA mini kits (QIAGEN®, France). Nucleic acid quantification was applied by NanoDrop 3300 (ND-3300, Thermo Scientific™, USA).

Partial 16S rRNA identification: The bacterial isolates were subjected to 16S rRNA gene sequencing using universal primers 27F (5′-AGRGTTTGATCMTGGCT CAG) and 1492R (5′-GGTTACCTTGTTACGACTT) as described previously (Holman *et al.*, 2015). The purified PCR products were sequenced using Genetic analyzer 3500, (Applied Biosystems, USA). 16S rRNA sequence homology searches were carried out using NCBI Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blas tn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

Detection of viral and bacterial pathogens: The genomic DNA was amplified for detection of suspected bacteria (*M. haemolytica* and *H. somni*) and the RNA for viruses (BVDV, BHV-1, BRSV, PI3V) using primers and PCR conditions as described previously (Table 1). (Angen *et al.,* 1998; Angen *et al.,* 2009; Horwood and Mahony, 2011; Klima *et al.,* 2014).

Table 1: Primers used in the present study

PCR target	Primer name	PCR primer sequence (5' to 3')	Amplicon size(bp)	Annealing temp	Reference(s)
Histophilus somni	HS453_F	5'-GAAGGCGATTAGTTTAAGAG-3'	400	55	Angen *et al.*, 1998
	HS860_R	5'-TTCGGGCACCAAGTRTTCA-3'		55	
M. haemolytica	MHlkt-int_F	5'-GTCCCTGTGTTTTCATTATAAG-3'	385	58	Angen *et al.*, 2009
	MHlkt-int_R	5'CACTCGATAATTATTCTAAATTAG-3'		58	
BVDV	RT_BVDVF	5'-AGCGAAGGCCGAAAAGAGGC-3'	312	58	Klima *et al.*, 2014
	RT_BVDVR	5'-CAACTCCATGTGCCATGTACAG-3'		58	
PI3V	RT_PI3VF	5'-CATAAGTGATCTAGATGATGATCC-3'	460	55	Klima *et al.*, 2014
	RT_PI3VR	5'-TTCATCTAGAATCTGAACTACTCC-3'		55	
BRSV	RT_BRSVF	5'-CAAACTAAATGACACTTTCAACAAG-3'	566	54	Horwood and Mahony, 2011 and Klima *et al.*, 2014
	N_BRSVR	5'-CATTTCATTCCTTAGTACATTGTTG-3'	422	63	Horwood and Mahony, 2011 and Klima *et al.*, 2014

Biochemical analysis of selected biomarkers: Proinflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-8) and PCT were analyzed using ELISA test kits (CUSABIO, Wuhan, Hubei, China). In addition, levels of NPT were determined in serum samples through competitive ELISA Kits (CUSABIO, Wuhan, Hubei, China). For both groups the serum Hp levels and SAA were tested using ELISA kits (Phase SAA kit, Tridelta Ltd., Ireland) according to the manufacturer's instructions.

Statistical analysis: JMP for Windows Version 5.1; SAS Institute, Cary, NC, USA (A statistical software program) was used for analyses of the data. MANOVA repeated measures on treatment and time was used to confirm the treatment efficacy. To assess the interactions and verification of time group interactions within group Wilks' Lambda test was used. In addition, one-way ANOVA with Tukey-Kramer HSD post-hoc multiple comparison tests were used to distinguish which group was statistically different from the others. The correlation of selected biochemical variables with response to treatment by different combination therapy was determined using Pearson correlation test coefficient.

RESULTS

Clinical appearance: Sixty-nine FL with *M. haemolytica* and/or *H. somni* exhibited various clinical signs of BRD (Table 2). FL that were apparently healthy with no isolated pathogenic bacteria or virus (n=20) were used as negative controls. Pneumonic FL have exhibited abnormal lung sound, cough and elevated (P<0.05) clinical parameters (heart rate, respiratory rate and body temperature) when compared with the control group (Table 2). The clinical signs of pneumonic feedlots were evaluated 7- days after treatment protocol with different antibacterial agents combined with non- steroidal anti-inflammatory drugs. According to their response to the treatment, the diseased feedlots were further categorized as i) responsive (n=49) and ii) non-responsive groups (n=20). Within the first 7-days after initiation of the therapy, the mean heart rate, respiratory rate, body temperature and abnormal lung sound were returned to the level compatible of that of the control group (n=49). However, the clinical signs (fever, abnormal lung sounds, cough and nasal discharge,) were still noticeable in 20 FL.

Bacterial and viral pathogen detection: *H. somni* and *M. haemolytica* were isolated from 69 nasopharyngeal swabs (27.6%). *H. somni* was isolated from 49 cases (19.6%) and *M. haemolytica* from 58 cases (23.2%). Coexistence of both bacteria was found in 38 cases (15.2%).

Table 2: The clinical sum scoring associated with *M. haemolytica* and *H. somini* infections in 69 feedlot calves compared with 20 healthy calves

Variables	Healthy calves (n=20)	Diseased calves (n = 69) *
Respiratory rate (Breath/min)	0	1.47±0.87
Rectal temperature (^{0}C)	0	1.68±0.73
Cough	0	1.62±0.78
Eye discharge	0	1.33±0.49
Nasal discharge	0	3.71±1.03
Eye droop	0	2.35±1.66

*P<0.05, significant differences between healthy calves and diseased calves. Each value represents (mean±SD).

Table 3: Correlation between responses to the treatment by various antibacterial agents combined with flunixin meglumine in calves naturally infected with *M. haemolytica* and *H. somini* infections

Variables	CEF	Tula	FFC	Tm
HP	0.750	0.002	0.020	0.008
SAA	0.333	0.003	0.022	0.462
IL-1β	0.7500	0.003	0.022	0.008
IL-8	0.333	0.003	0.022	0.008

The final obtained data are the *P* value using Pearson correlation test coefficient. CEF, Ceftiofur Na, Tula, Tulathromycin, FFC, florfenicol, Tm, tilmicosin. Differences between means at P<0.05 were considered significant.

Table. 4: Correlation between responses to the treatment of various antimicrobial agents combined with Phenylbutazone in calves naturally infected with *M. haemolytica* and *H. somini* infections

Variables	CEF	Tula	FFC	Tm
HP	0.050	0.132	0.048	0.083
SAA	0.225	0.299	0.034	0.750
IL-1β	0.001	0.151	0.048	0.083
IL-8	0.0003	0.132	0.048	0.083

The final obtained data are the *P* value using Pearson correlation test coefficient. CEF, Ceftiofur Na, Tula, Tulathromycin, FFC, florfenicol, Tm, tilmicosin. Differences between means at P<0.05 were considered significant.

The 16S rRNA gene sequences from each isolate was subjected to BLAST analysis and representative sequences were submitted to GenBank with accession no MG725885, MG725884.1, MG725883, MG725826.1, MG725726. All isolates were negative for BVDV, BHV-1, BRSV, and PI3V by PCR examination.

Cytokines and APPs in sera: In this study, biochemical investigation of selected cytokines and APPs in sera of pneumonic FL revealed higher levels of examined inflammatory cytokines (IL-1β, IL-8, IFN- γ, and TNF-α) and selected APPs (Hp, and SAA) compared to those healthy control animals (Fig. 1, 5 and 6). Substantial reduction (P<0.05, Wilks' Lambda test for drug x time interaction, P<0.0001) in the serum values was observed for all examined biomarkers in all treated FL after 7 days of treatment (Fig. 1, 5 and 6). Interestingly, the levels of IL-8, IFN- γ & Hp in diseased FL treated with the combination therapy of various antibacterial & non-

steroidal anti-inflammatory agents returned similar to the levels of that of control group (Fig. 1, 4 and 6). Similarly, the levels of IL-1β in FL treated with PBZ returned to normal state (Fig. 3a). In contrast, no such state of returning to normal levels was observed for SAA in all treated FL (Fig. 2). In this study we demonstrate the selective inhibitory effect of administrated non-steroidal anti-inflammatory agents either FM or PBZ on the production of examined cytokines and AAPs in FL with BRD.

Correlation of selected biochemical variables with response to treatment by different combination therapies: In this study, the responses to treatment by FM and PBZ correlated with the production of cytokines in sera & APPs sera level. FL treated with FM in combination with Tula, FFC, and Tm showed strong significant positive correlations (P<0.05) between the sera levels of Hp, IL-1β, and IL-8 and response to the treatment (Table 3). Moreover, administration of PBZ combined with FFC, and Tm showed a strong significant positive correlation (P<0.05) between the sera levels of Hp, IL-1β, and IL-8 and response to the treatment (Table 4). On contrary, SAA levels in FL treated with either FM or PBZ combined with Tm showed a non- significant positive correlation (P>0.05)

in response to the treatment (Tables 3 and 4). In addition, a non- significant positive correlation (P>0.05) was observed between all selected biochemical variables and response to the treatment in FL treated with either FM combined with CEF or PBZ in combination with Tula (Tables 3 and 4). On the other hand, treatment by CEF in combination with PBZ caused a strong significant positive correlation (P<0.05) between the sera levels of IL-1β, and IL-8 and response to the treatment (Table 4). Of note, the only combination therapy that showed a positive correlation (P<0.05) between the sera level of SAA and response to the treatment was the PBZ and FFC combination (Table 4). These results indicated the efficacy of FFC and Tm antimicrobial agents for the treatment of FL with BRD when administrated with either FM or PBZ. However, Tula is preferable to administrate in combination with FM for treatment of FL with RD. In particular, we observed a further decrease in the sera levels of Hp, IL-1β, and IL-8 in FL treated with either FM combined with Tula, FFC, and Tm or PBZ combined with FFC, and Tm, indicating the response to the treatment. Therefore, the sera level of SAA can be used as an indicator to response to the treatment in FL with BRD when FFC is administrated in combination with PBZ.
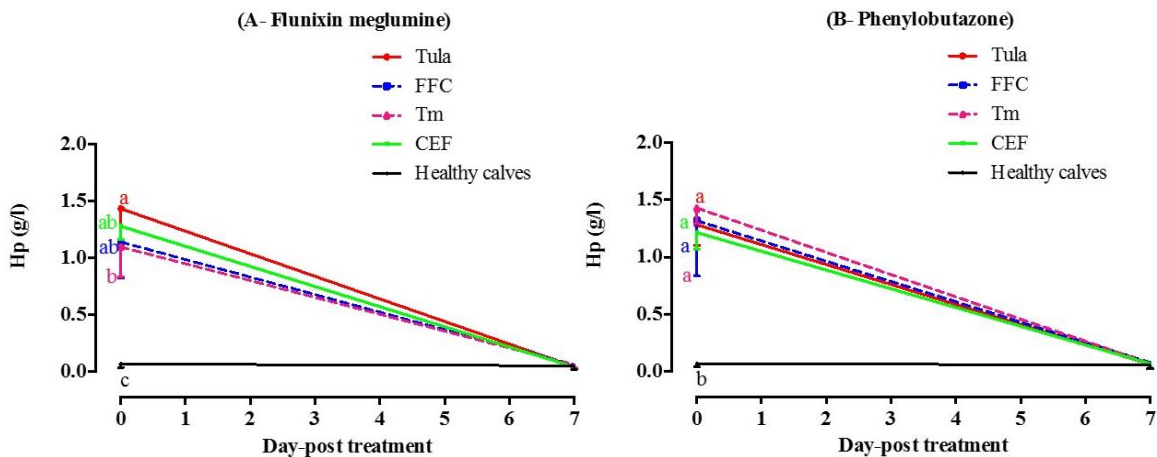

Fig. 1: Serum concentration of haptoglobin (HP) in feedlot calves naturally infected by *M. haemolytica* and *H. somni* and those treated with various antimicrobial agents combined with either flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time.
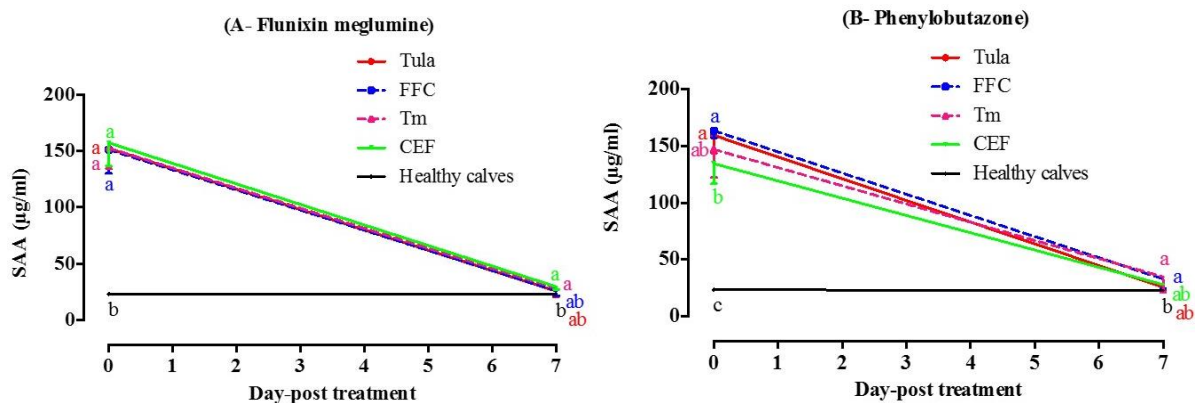

Fig. 2: Serum concentration of serum amyloid A (SAA) in feedlot calves naturally infected by *M. haemolytica* and *H. somni* and those treated with different antibacterial agents combined with either flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time.
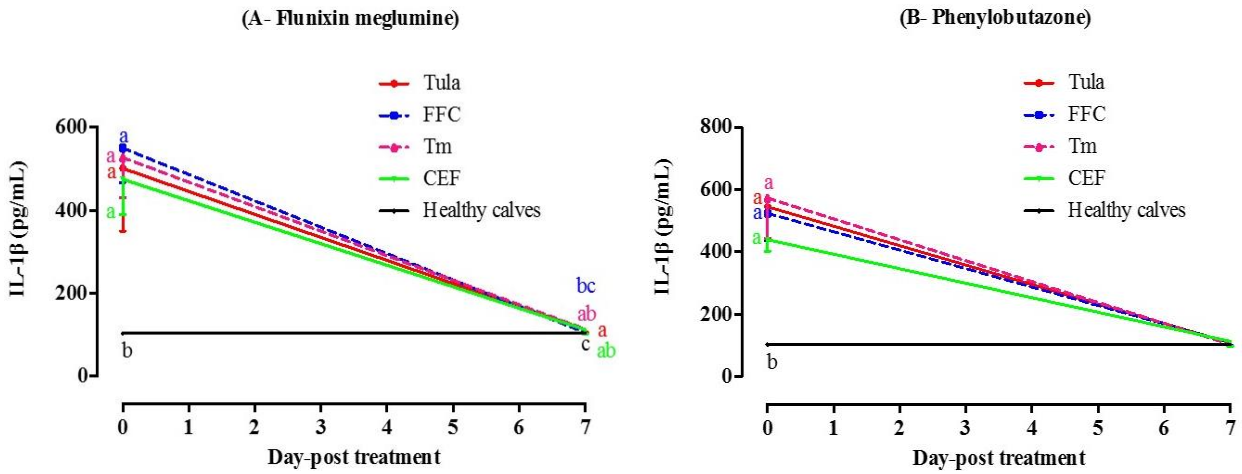
Fig. 3: Serum concentration of interleukin- I (IL-1β) in feedlot calves naturally infected by *M. haemolytica* and *H. somni* and those treated with different antimicrobial agents combined either with flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time interaction, P<0. 0001. Tula, tulathromycin; FFC, florfenicol; Tm, tilmicosin; CEF, Ceftiofur.
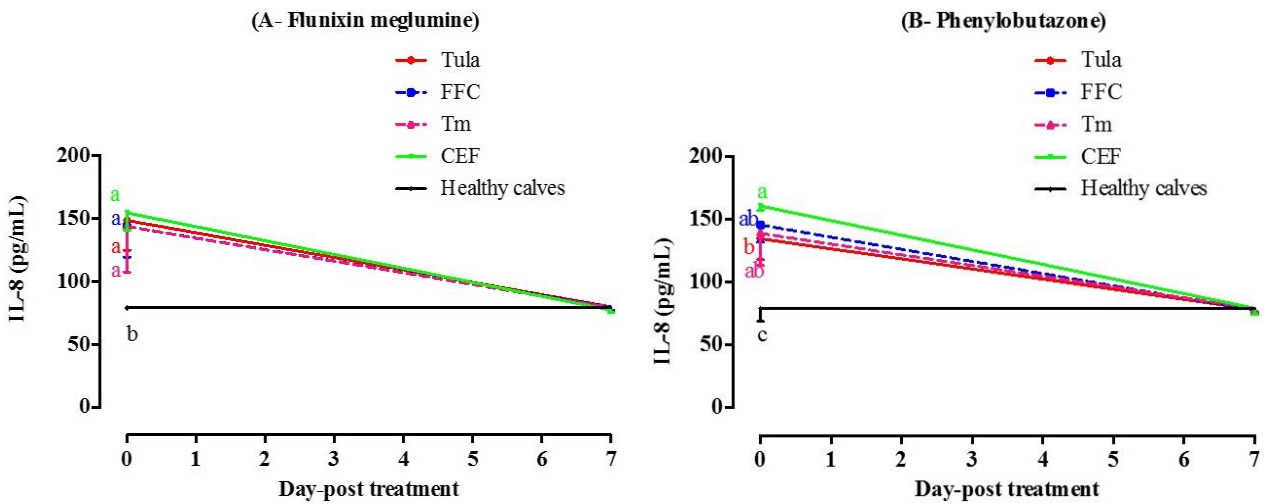

Fig. 4: Serum concentration of interleukin- 8 (IL-8) in feedlot calves naturally infected by *M. haemolytica* and *H. somini* and those treated with different antimicrobial agents combined either with flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time interaction, P<0. 0001. Tula, tulathromycin; FFC, florfenicol; Tm, tilmicosin; CEF, Ceftiofur.
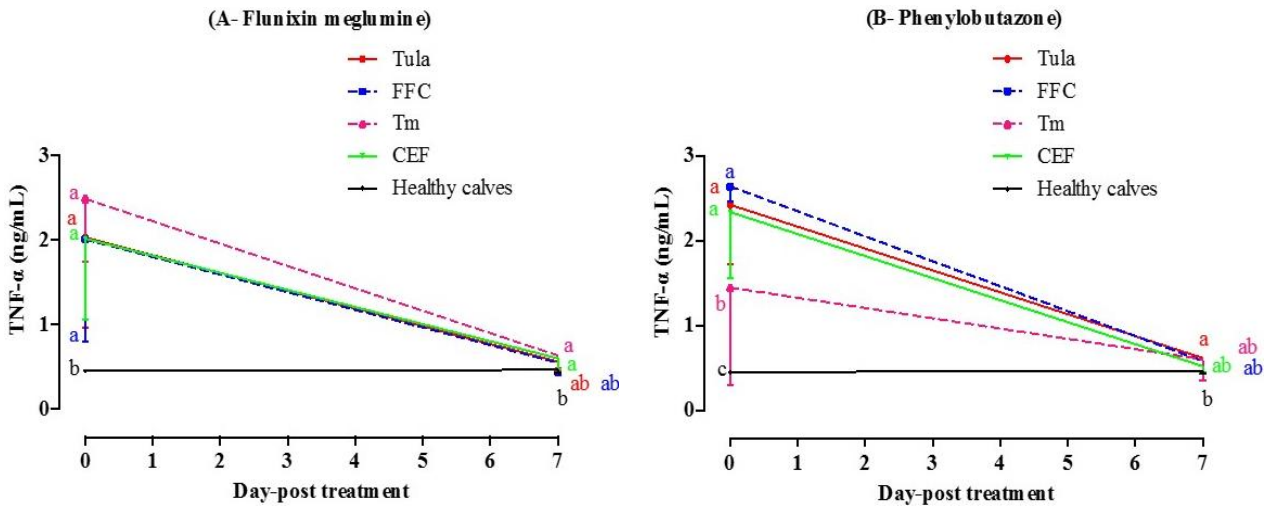

Fig. 5: Serum concentration of tumor necrosis factor- α (TNF-α) in feedlot calves naturally infected by *M. haemolytica* and *H. somni* and those treated with different antimicrobial agents combined either with flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time interaction, P<0. 0001. Tula, tulathromycin; FFC, florfenicol; Tm, tilmicosin; CEF, Ceftiofur.
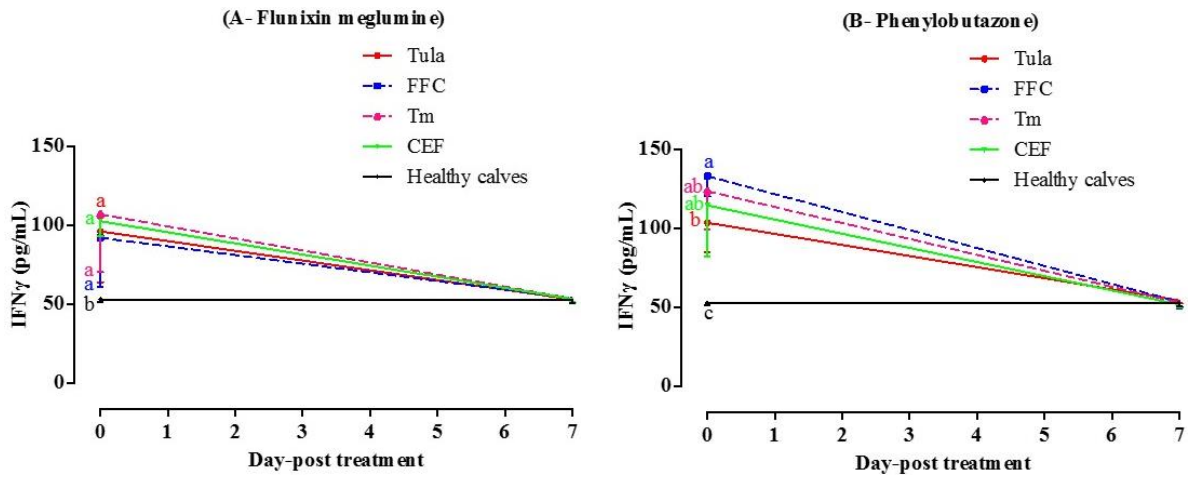
Fig. 6: Serum concentration of interferon gamma (IFNγ) in feedlot calves naturally infected by *M. haemolytica* and *H. somni* and those treated with different antimicrobial agents combined with either flunixin meglumine or Phenylbutazone. (a) Flunixin meglumine. (b) Phenylbutazone. Points with different superscript letters are statistically significantly different (P<0.05). Each value represents (mean±SD). MANOVA fit, P<0.0001. Wilks' Lambda test for drug x time interaction, P<0.0001. Tula, tulathromycin; FFC, florfenicol; Tm, tilmicosin; CEF, Ceftiofur.

DISCUSSION

To the best of our knowledge, this is the first study to address the correlation between the serum levels of APPs and cytokines in response to treatment by various antimicrobial agents in FL with BRD. Recently, we reported the selective inhibitory effect of tulathromycin when administered in combination with NSADs on the secretion of cytokines in pneumonic calves (Rizk *et al.*, 2017). However, we then did not explore whether such selective inhibitory effect was unique to the NSADs and tulathromycin combination only or to the NSAD combination with other antimicrobial agents. Therefore, in this study this selective effect was examined.

In this study, increased levels of all examined cytokines (TNF-α, IL-1β, IL-8 and IFNγ) and APPs (Hp, and SAA) were detected in FL infected with *H. somni* and *M. haemolytica*. In addition, we showed that higher levels of IL1β, IFNγ, TNF-α, and Hp were perceived in calves that suffered from respiratory diseases (Rizk *et al.*, 2017). Consistent to our data, others reported similar results for many infections and inflammations in cattle (Gruys *et al.* 2005) and in boar (Reeth and Nauwynck 2000). Increased serum levels of proinflammatory cytokines have previously been detected in calves or steers experimentally challenged with BVDV and/or *M. hemolytica* (Burciaga-robles *et al.,* 2010).

Seven days after treatment of the diseased FL by a combination therapy of various antibacterial agents and non-steroidal anti-inflammatory agents, substantial inhibition (P<0.05) in the serum levels of all selected biochemical variables was observed. Similar decrease in the expression of TNF-α was previously observed in macrophages of mice after treatment with aspirin (Shackelford *et al.*, 1997). Others also reported the effect of FM on IL-1 β, TNF-α, and IL-10 on mice with experimentally induced endotoxemia (Yazar *et al.*, 2007). On contrary, no significant alterations were detected in the production of IL-1 &TNF- α in human after NSADs (naproxen and ibuprofen) administration (Hartman *et al.*, 1993). The dissimilar mode of action of diverse NSADs beside the variety in the experimental models and animal

species that used in each study may interpret such inconsistencies in the levels of different cytokines in different studies.

It is largely accepted that meloxicam is efficient in standardization of TNF-α levels in both bronchoalveolar lavage and blood of calves with experimentally induced pneumonia (Bednarek *et al.*, 1999). In this study, normalization of the examined cytokines secretion and APPs levels was observed in IL-8 and Hp in all treated feedlot calves and in the IL-1β sera level in feedlots treated with PBZ. Consistent with our findings, treatment of pneumonic buffalo calves with Tula and FM within the same inoculation period had previously returned the serum level of IL-1β to a normal (Rizk *et al.*, 2017). Our findings together with those recently reported (Rizk *et al.*, 2017) indicate that NSADs have selective influence on the APR in pneumonic calves. The obvious inhibition in the secretion of TNF-α without normalization its serum level recorded in this study may be considered beneficial for the pneumonic FL. Another *in vitro* study has demonstrated the positive impact of *M. haemolytica* and *H. somni* in the secretion of TNF-α in the cultures of bovine bronchoalveolar lavage cells (Bienhoff *et al.*, 1992). The stimulation of TNF-α production-causes enhancement in both collagenase activity, and prostaglandin E_2 (PGE_2) secretion with remodeling of connective tissue during inflammatory conditions (Bienhoff *et al.*, 1992; Bednarek *et al.*, 1999). Therefore, TNF- α has a protective role during bacterial & viral challenges (Vassali, 1992). Consequently, the significant inhibitory effect of the administered combination therapy on the secretion of TNF-α in the serum of pneumonic feedlots deprived of normalization may well be considered as an additional value to such anti-inflammatory drugs in the treatment protocol of pneumonic FL.

Conclusions: The results of this study demonstrate the selective potent inhibitory influence of administered non-steroidal anti-inflammatory agents either FM or PBZ on the production of cytokines and APPs (APR) in FL with BRD. Our data showed the efficacy of FFC and Tm antimicrobial agents for the treatment of FL naturally infected with *BRD*

when administrated with either FM or PBZ. However, we also demonstrated that Tula was preferable for administration in combination with FM for the treatment of FL with RD. More importantly, monitoring the sera levels of Hp, IL-1β, and IL-8 in FL treated with either FM combined with Tula, FFC, and Tm or PBZ combined with FFC, and Tm was effective in predicting the disease prognosis.

 Authors contribution: We, designed the study, analyzed the sera samples and wrote the manuscript; MR, performed the statistical analysis and wrote the manuscript; MF performed the microbiological work in this study; HM critically reviewed the manuscript and shared in writing the manuscript; MK; shared in interpretation of antibiotic and anti-inflammatory effects. All authors interpreted the data, critically revised the manuscript.

REFERENCES

Angen O, Ahrens P and Tegtmeier C, 1998. Development of a PCR test for identification of *Haemophilus somnus* in pure and mixed cultures. Vet Microbiol 63:39-48.

Angen, Ø, Thomsen J, Larsen LE, *et al.*, 2009 Respiratory disease in calves: microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. Vet Microbiol 137:165-71.

Bednarek D, Szuster-Ciesielska A, Zdzisińska B, *et al.*, 1999. The effect of steroidal and non-steroidal anti-inflammatory drugs on the cellular immunity of calves with experimentally induced local lung inflammation Vet Immunol Immunopathol 71:1-15.

Bednarek D, Zdzisińska B, Kondracki M, *et al.*, 2003. Effect of steroidal and non-steroidal anti-inflammatory drugs in combination with long-acting oxytetracycline on non-specific immunity of calves suffering from enzootic bronchopneumonia. Vet Microbiol 96:53-67.

Benchaoui HA, Nowakowski M, Sherington J, *et al.*, 2004. Pharmacokinetics and lung tissue concentrations of tulathromycin in swine J Vet Pharmacol Ther 27:203-10.

Bienhoff SE, Allen GK and Berg JN, 1992. Release of tumor necrosis factor-alpha from bovine alveolar macrophages stimulated with bo-vine respiratory viruses and bacterial endotoxins Vet Immunol Immunopathol 30:341-57.

Burciaga-robles LO, Step DL, Krehbeil CR, *et al.*, 2010. Effects of exposure to calves persistently infected with bovine viral diarrhea virus type 1b and subsequent infection with *Mannheima haemolytica* on clinical signs and immune variables: Model for bovine respiratory diseases via viral and bacterial interaction. J Anim Sci 88:2166-78.

El-Deeb WM and Buczinski S, 2015. The diagnostic and prognostic importance of oxidative stress biomarkers and acute phase proteins in Urinary Tract Infection (UTI) in camels. Peer J 3:e1363; DOI 107717/peerj1363.

 El-Deeb WM and El-Moslemany A and Salem MAM, 2017. Cardiac troponin I and immune-inflammatory response in horses with strangles. J Equine Vet Sci 51:18-23.

El-Deeb WE, Elsohaby I, Fayez M, *et al.*, 2020. Use of procalcitonin, neopterin, haptoglobin, serum amyloid a and proinflammatory

cytokines in diagnosis and prognosis of bovine respiratory disease in feedlot calves under field conditions. Acta Tropica 204:105336. DOI information: 10.1016/j.actatropica.2020.105336.

El-Deeb WM and El-Moslemany AM, 2016a. The diagnostic accuracy of acute phase proteins and proinflammatory cytokines in sheep with pneumonic pasteurellosis. Peer J 2016 Jul 19;4:e2161 doi: 107717/peerj2161 eCollection 2016.

El-Deeb WM and El-Moslemany AM, 2016b. The diagnostic accuracy of acute phase proteins in cases of urinary tract infections in dairy cattle. Japanese J Vet Res 64:57-66.

El-Deeb WM and Iacob O, 2012. Serum acute phase proteins in control and Theileria annulate infected water buffaloes (*Bubalus bubalis*). Vet Parasitol 23:12-8.

El-Deeb WM and Tharwat MA, 2015. Lipoproteins profile, acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep with pneumonic pasteurellosis. Compar Clin Pathol 24:581-8.

El-Deeb WM, Fouda TA and El-Bahr SM, 2014. Clinicobiochemical investigations of Partuberclosis of camels in Saudi Arabia: Proinflammatory cytokines and oxidative stress markers. Pak Vet J 34:484-8.

Gruys E, Toussaint MJM, Niewold TA *et al.*, 2005. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci 6:1045-56.

Hartman DA, Ochalski SJ and Carlson RP, 1993. The effect of anti-inflammatory and anti-allergic drugs on the release of IL-1 and TNF-α in human whole blood assay Agents Actions 39:C70-2.

Holman DB, Timsit E and Alexander TW, 2015. The nasopharyngeal microbiota of feedlot cattle. Sci Rep 5:15557.

Horwood PF and Mahony TJ, 2011. Multiplex real-time RT-PCR detection of three viruses associated with the bovine respiratory disease complex. J Virol Methods 171:360-3.

Joshi V, Gupta VK, Bhanuprakash AG, *et al.*, 2018. Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. Microb Pathog 116:33-7.

Klima CL, Zaheer R, Cook SR, *et al.*, 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J Clin Microbiol 52:438-48.

Lekeux P, 1996. Use of antiinflamatory drugs in the treatment of bovine respiratory disease complex In Proceedings of the XIX World Buiatric Congress, Edinburgh pp:45-9.

Reeth KV and H Nauwynck, 2000 Proinflammatory cytokines and viral respiratory disease in pigs. Vet Res 31:187-213.

Rizk MA, Mahmoud ME, El-Sayed SAE, *et al.*, 2017. Comparative therapeutic effect of steroidal and non-steroidal anti-inflammatory drugs on pro-inflammatory cytokine production in water buffalo calves (*Bubalus bubalis*) naturally infected with bronchopneumonia: a randomized clinical trial. Trop Anim Health Prod Dec 49:1723-31.

Shackelford RE, Alford PB, Xue Y, *et al.*, 1997. Aspirin inhibits tumor necrosis factor gene expression in murine tissue macrophages. Mol Pharmacol 52:421–9.

Slee KJ and Stephens LR, 1985. Selective medium for isolation of Haemophilus somnus from cattle and sheep. Vet Rec 23:215-7.

Van de Weerdt ML and Lekeux P, 1997. Modulation of lung inflammation in the control of bovine respiratory disease. Bovine Pract 31:19-30.

Vassali P, 1992. The pathophysiology of tumor necrosis factor. Annu Rev Immunol 10:411-52.

Woolums AR, Karisch BB, Frye JG, *et al.*, 2018. Multidrug resistant Mannheimia haemolytica isolated from high-risk beef stocker cattle after antimicrobial metaphylaxis and treatment for bovine respiratory disease. Vet Microbiol 221:143-52.

Yazar E, Er A, Uney K, *et al.*, 2007. Effect of flunixin meglumine on cytokine levels in experimental endotoxemia in mice. J Vet Med A Physiol Pathol Clin Med 54:352-5.