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Original Article

Efficacy of 'Actovet-CRD' a Novel Herbal Formulation as Prophylactic and Therapeutic Agent for CRD Complex in Poultry

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ABSTRACT

Actovet CRD (A.CRD) is a blend of ayurvedic herbs (*Glycorrhyza glabra*, *Adhatoda vasika*, *Pipper longum*, *Abis Webbiana*, *Azadirachta indica*, *Curcuma longa etc)* which have known therapeutic effect on respiratory system and other systemic diseases with positive effect to boost immunity. To find the efficacy of A.CRD and to optimize its level of supplementation against the chronic respiratory disease (CRD) three age groups of broilers viz., 0 day, 14 day and 28 day representing the age groups of 0-2 weeks (T1), 2-4 weeks (T2) and >4 weeks (T3) of age respectively were isolated for the experiment from a large size group of birds from each of five farmers. The results of the trial indicated that A.CRD improved the body weight gain, survivability, haemoglobin, PCV, total protein levels significantly ($P \leq 0.05$). The symptoms of the CRD in the infected birds subsided within a week period of treatment. The birds stopped gasping, rales and other symptoms of the CRD within 4-7 days of treatment at all therapeutic dosage levels. The results indicated that the birds of age group T1, T2 and T3 should be supplemented with A.CRD at the rate of 10, 20 and 40 ml/100 birds respectively. The birds did not show any symptoms of drowsiness, off feed which is usually observed in antibiotic treatment. The results showed that A.CRD can replace antibiotic therapy. Hence, this herbal formulation can be used for prevention and treatment of CRD and a suitable supplement for organic poultry farming.

Key words: Actovet-CRD, Antibiotic Resistance, Chronic Respiratory Disease, Herbal Formulation, Organic Poultry Farming, Poultry.

INTRODUCTION

Presently the poultry industry suffers from heavy losses due to mortality, reduced egg production, sub clinical infections which not only cause stunted production, but also would find no market. Moreover, unannounced outbreaks of economically important diseases render complete destocking of the poultry farm including parent stock and its products like egg, meat etc.

When the health department notices such incidences of disease outbreaks, they suggest closure/relocate the poultry farms. Chronic Respiratory Disease (CRD)/avian mycoplasmosis is of high economic importance as this causes heavy production loss and mortality of the birds. Avian Mycoplasmosis is caused principally by three species of Mycoplasma organisms. Mycoplasma gallisepticum (MG), Mycoplasma synoviae (MS) occur in chickens and turkeys. This is usually complicated by other secondary infections due to *Streptococcus, E.coli*, etc and other sub clinical infections. CRD not only involves respiratory system but also reproductive system in the layers. Broilers and layer chicks in the age group of 4-8 weeks are affected most (Ganti and Rama Rao, 2004). Pure breed flocks it appears to be genetic as it seems to affect only some strains and not others. Various antibiotics are continuously used as antibiotics as feed additives pose the threat of their accumulation in the viscera of the birds and passing on to the end user- the consumers. Also, the drug resistance may be shown to these antibiotics which needs constant search for upgraded genre/generation of antibiotics. Poultry litter contains antimicrobial residues and resistant bacteria (like *Escherichia coli)*; when applied as fertilizer, the level and effects of these pharmaceuticals and antimicrobial-resistant bacteria in the environment are of concern. *E. coli* in major would be resistant to many antibiotics. Resistance to β-lactam antibiotics (amoxicillin, ceftiofur), tetracyclines, and sulfonamides was the most prevalent. Studies have concluded that broiler litter is a source of antimicrobial residues and represents a reservoir of multiple antibiotic-resistant *E. coli* (Furtula *et al.*, 2010). Pathogens and indicator bacteria tested were found to be resistant to multiple antibiotics. Data suggest that microbial contamination of litter should be reduced or eliminated prior to reutilization to minimize environmental health risks

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related to transfer of antibiotic-resistant bacteria to humans or other animals (Kelley, 1998).

The EU Commission banned the use of all antibiotic feed additives classed as growth promoters in the EU against the advice of its own Scientific Committee on Animal Nutrition (SCAN, 1996; SCAN, 1998). Also, due to high cost of conventional medicines, vaccines, they are usually out of reach of the small-scale farmers (Okitoi, 2007).

Hence, the need of the hour is to find a suitable herbal drug which constantly prevents birds from the threat of new infection, alleviation of sub clinical infections and immuno booster in a most natural way.

MATERIALS AND METHODS

A. CRD is a blend of herbs from the ancient Ayurveda, *viz.*, *Glycorrhyza glabra* (antiviral, expectorant, carminative property and masks bitter taste) (Susan and Barbara, 2007; Thorne research, 2005), *Adhatoda vasika* (therapeutic effects against respiratory infections) (Dorsch, 1991; Narimanian, 2005), *Pipper longum*, *Abis webbiana*, *Azadirachta indica* (known for their medicinal properties against respiratory and other diseases), (Bonsu *et al*, 2012, Susan and Barbara, 2007), *Curcuma longa* (antiviral, mucolytic, antitussive properties) are processed with synergeistic herbs. These herbs have inhibitory effect on various microorganisms such as Staphylococcus, Salmonella, E.coli etc. (Watai, 2003).

An experiment was conducted in five different commercial layer poultry farms to study the efficacy of the formulation 'Actovet-CRD' (a formulation of *Celest Pharma labs Pvt. Ltd*, Devarahosahally, Nelamangala taluk, Bangalore, India) as a prophylactic and therapeutic agent against the respiratory diseases which includes symptomatic relief from CRD complex, general health of the birds, and as immune booster to counteract other secondary infections.

Five commercial poultry farms in and around Chikmagalur district, Karnataka state were identified. The farms maintained same breeds of broiler chicks with comparable body weights at different age groups. Three age groups of 0 day, 14 day and 28 day representing the age groups of 0-2 weeks (T1), 2-4 weeks (T2) and >4 weeks (T3) of age respectively were isolated for the experiment from a large size group of birds from each farmer. A.CRD was added at 3 levels of inclusions, for T1 (0-2 weeks of age group) viz., 10, 15, 20 ml/100 birds and they were termed as TA1, TB1 and TC1 respectively. Similarly, T2 (2-4 weeks of age) received 20, 25 and 30 ml/100 birds termed as TA2, TB2 and TC2 respectively. T3 (>4 weeks of age) was treated with 30,35 and 40 ml/100 birds and termed as TA3, TB3 and TC3 respectively through drinking water. Each treatment consisted of 100 birds for experiment and similar numbers of birds were kept as control, namely, C1, C2 and C3 for age groups T1, T2 and T3 respectively.

All the birds received same poultry feed manufactured according to the standard protocols. The body weights of the birds, blood sample collection was done every week. The birds were bled via wing veins using sterile 21G x 1½" (0.8 x 40 mm) needles and syringes into sterile vials containing anticoagulant (ethylene diamine tetra acetic acid; EDTA) and without anticoagulant separately. Decanted sera from the coagulated blood samples were saved by freezing at -18ºC until analyzed for different parameters.

The birds were observed for body weight, survivability, clinical signs, hematological parameters like hemoglobin, packed cell volume (PCV), serum glucose, total protein, albumen, globulin, A:G ratio, serum creatinine, cholesterol, SGPT, SGOT, farmer's satisfaction etc. The data was recorded and was analyzed according to the standard protocols of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The area of the present study is located in malenad region (western ghats) receiving high annual rainfall of 922 to 3695 mm and humidity (Rudraswamy *et al.*, 2012) which precipitates respiratory disorder in birds including CRD. The symptoms included off feed, gasping for breath, isolation from the flock, sneezing, rattling, water gargling/flowing sound in the early morning hours near the flock (as the birds face difficulty in respiration during this humid period), gaseous or beaded exudates in air sacs and tracheal mucosa, thickened pneumonic areas in the lungs on PM examination and others symptoms of CRD (Jagadish Prasad, 2005). The therapy to this disease can be comparable with the use of antibiotics in poultry feed (Olga and Henry, 1959). These birds were treated with A.CRD according to the prototcol.

Effect on Growth

The results of the experiment for the age groups of 0-2 weeks have shown that there was significant change in the growth of the birds as indicated by their body weight (table 1).

Body weights (g) recorded for control, TA1, TB1 and TC1 were 410, 475, 485, 510 respectively ($P \leq 0.05$). The body weights of the birds TA1 was significantly higher than control but was not different with further level of supplementation of A.CRD. Hence, T1 age group birds should be supplemented at 10 ml/100 birds. In T2 group body weights (g) recorded for control, TA2, TB2 and TC2 were 1065, 1290, 1350 and 1380 respectively. The body weights of the birds TA2 was significantly higher than control but was not different with further level of supplementation of A.CRD. Hence, T2 age group birds should be supplemented at 20 ml/100 birds. The body weight (g) of birds in T3 recorded for control, TA3, TB3 and TC3 were 1850, 2060, 2120 and 2345 respectively. The body weights of the birds TC3 was significantly higher than control and further level of supplementation of A.CRD. Hence, it is recommended that the 4-6 weeks of birds should be supplemented with A.CRD at the rate of 40 ml/100 birds. Similar results were obtained when *Azadirachta indica* and *Rawolfia vomitoria* decoctions fed to broilers replacing antibiotic and coccidiostats showed positive effect on body weights (Zanu *et al*., 2011).

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Table 1. Body weight of birds (g) before and after the treatment with Actovet CRD (*)

Age (Weeks)	Parameters	BW (g) of control	Body weight of the experimental birds (g)		
			TA1 (10 ml/100 birds)	TB1 (15ml/100 birds)	TC1 (20 ml/100 birds)
T1 (0-2 weeks of age)	Initial BW	33±2.8	32±3.5	32±1.9	33±4.3
	Final BW	410±13.2[a]	475±12.9[b]	485±16.3[b]	510±22.9[b]
	Change in BW during the experiment	377[a]	443[b]	453[b]	477[b]
	Difference in BW of experimental with control group		66	76	100
T2 (2-4 weeks of age)			TA2 *(20ml/100 birds)*	TB2 *(25ml/100 birds)*	TC2 *(30ml/100 birds)*
	Initial BW	415±18.1	420±23.8	420±15.9	415±19.1
	Final BW	1065±23.8[a]	1290±29.5[b]	1350±18.9[b]	1380±24.1[b]
	Change in BW during the experiment	650[a]	870[b]	930[b]	965[b]
	Difference in BW of experimental with control group		220	280	315
T3 (>4 weeks of age)			TA3 *(30ml/100 birds)*	TB3 *(35ml/100 birds)*	TC3 *(40ml/100 birds)*
	Initial BW	1180±18.9	1190±21.5	1185±16.8	1180±24.6
	Final BW	1850±29.4[a]	2060±24.5[b]	2120±16.9[b]	2345±24.3[c]
	Change in BW during the experiment	670[a]	870[b]	935[b]	1165[c]
	Difference in BW of experimental with control group		200	265	495

* The values in a row are statistically different from each other (P≤0.05).

Table 2. Survivability (%) of birds during the experiment

Age (Weeks)	Control	Experimental birds		
T1 (0-2 weeks of age) *		TA1	TB1	TC1
	84±2.8[a]	91±1.9[b]	93±1.3[b]	93±2.1[b]
T2 (2-4 weeks of age) *		TA2	TB2	TC2
	85±3.1[a]	91±1.8[b]	92±2.5[b]	92±0.6[b]
T3 (>4 weeks of age) *		TA3	TB3	TC3
	85±2.2[a]	90±1.4[b]	90±1.5[b]	93±0.8[b]

* -The values in a row are statistically different from each other (P≤0.05).

Clinical symptoms and Survivability

The symptoms of the CRD in the infected birds subsided within a week period of treatment. The birds stopped gasping, rhales and other symptoms of the CRD within 4-7 days of treatment at all therapeutic dosage levels. Similar results were obtained when the neem leaf meal was included in the ration of broiler chickens controlled coccidiosis, worm infestation and respiratory infections when compared with the control (Bonsu *et al*., 2012). The alleviation of respiratory symptoms is attributed to its herbal ingredients like *Azadirachta indica* which inhibited mycelium *in vitro* (Alexandre *et al.*, 2005 and Lakshmanan *et al*,. 1990); *Adhatoda vasica* having proven therapeutic effects against cold, cough, respiratory distress and other symptoms in Ayurveda (Dhuley, 1999; Sharma, 2000) and others.

The time required for different experimental group to return from morbid stage to healthiness varied upon the flock and age group. The birds remained active and there were no symptoms of drowsiness, off feed which is usually exhibited during dosing antibiotic at prophylactic or therapeutic levels.

The survivability % of the chicks of T1 age group for control, TA1, TB1 and TC1 were 84, 91, 93 and 93; T2 age group for control, TA2, TB2 and TC2 were 85, 91, 92 and 92 and for T3 age group for control, TA3, TB3 and TC3 were 85, 90, 90 and 93 respectively (P≤0.05). This suggests that higher survival rate is obtained in A.CRD is supplemented at 10, 20 and 40 ml/100 ml birds for the T1, T2 and T3 age groups respectively. The results were in accordance with the findings of Mujeeb Athar *et al* (2009) where the broilers had higher survival rate when dosed with polyherbal drug when compared to control.

Haematology

Haemoglobin is the indicator for general health and nutrition status (Jerry Kaneko, 1999). Haemoglobin (g%) and PCV (%)along with total protein levels (table 3,4,5) were low for the control in all the age group which may be due to low feed intake,

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stress on birds, respiratory distress and secondary infections.

Haemoglobin levels enhanced in treatment groups and were significantly higher in TA1, TA2 and TC3 in T1, T2 and T3 age groups respectively. Other parameters like blood glucose, creatinine, cholesterol, SGPT and SGOT were within the normal range (Jerry Kaneko, 1999).

Post mortem examination conducted in dead and sacrificed birds revealed varying degrees of mucous in the nasal sinuses, trachea, lungs and air sacs with catarrhal inflammation; mucous either watery or like cheese (Ganti and Rama Rao, 2004) especially in control birds.

The results showed that A.CRD can replace antibiotic therapy. Prior research also have proven that herbal formulations are equally effective as antibiotics which was evidenced by a study where broilers were dosed with Enrofloxacin and polyherbal formulation (AV/CRP/11) which showed equal efficacy in treating respiratory and other infections of poultry (Mujeeb Ather *et al*, 2009).

Table 3. Haematological parameters of T1 age group birds

Parameters	**Control**	**Treatments**			**Significant[1]**
		TA1	TB1	TC1	
Hb (g/dl)	8.4±0.12[a]	10.0±0.14[b]	10.1±0.28[b]	10.8±0.22[b]	Improvement in general health condition of the birds enhanced the value of Hb and PCV
PCV (%)	26±2.35[a]	31±4.51[b]	32±3.41[b]	32±1.55[b]	
ALT (U/L)/SGPT	4.39±0.31	4.58±0.19	4.93±0.53	4.69±0.44	
AST (u/L) /SGOT	159±11.68	164±10.91	172±19.30	170±15.83	
Glucose (mg/dl)	163±21.44	159±23.61	165±19.11	168±21.86	
Creatinine (mg/dl)	1.41±0.22	1.17±0.31	1.01±0.21	1.14±0.23	
Total protein (g/dl)	4.02±0.15	4.59±0.21	4.63±0.13	4.69±0.22	
Albumen (g/dl)	1.82±0.21	1.95±0.09	1.98±0.11	1.96±0.08	
Globulin (g/dl)	2.2±0.09	2.64±0.11	2.65±0.13	2.73±0.16	
A:G ratio	1.21±0.06	1.35±0.15	1.34±0.09	1.39±0.08	
Cholesterol (mg/dl)	121±11.86	136±21.03	132±30.28	129±22.41	

[1]The values in a row in italics are statistically different from each other (P≤0.05). Hb-Haemoglobin; PCV-Packed cell volume; ALT-Alanine amino transferase; SGPT-serum glutamic pyruvic transaminase; AST-Aspartate amino transferase; SGOT-Serum glutamic pyruvic transaminase and A:G ratio-Albumen:Globulin ratio

Table 4. Haematological parameters of T2 age group birds

Parameters	**Control**	**Treatments**			**Significant[2]**
		TA2	**TB2**	**TC2**	
Hb (g/dl)*	7.68±0.14[a]	9.28±0.25[b]	9.68±0.55[b]	10.2±0.32[b]	Improvement in general health condition of the birds enhanced the value of Hb
PCV (%)	26±2.40	29±3.46	30±4.81	30±3.05	
ALT (U/L)/SGPT	3.89±0.28	4.11±0.65	4.25±0.63	4.41±0.57	
AST (u/L) /SGOT	143±23.52	151±18.38	153±21.20	160±14.01	
Glucose (mg/dl)	138±13.95	151±16.45	159±19.12	163±24.18	
Creatinine (mg/dl)	1.25±0.12	1.31±0.30	1.43±0.35	1.31±0.26	
Total protein (g/dl)*	4.09±0.20	4.61±0.19	4.67±0.43	4.69±0.19	No drowsiness and off feed conditions and improvement in nutritional status of birds
Albumen (g/dl)	1.88±0.22	2.01±0.19	2.09±0.16	2.1±0.08	
Globulin (g/dl)	2.21±0.21	2.6±0.11	2.58±0.15	2.59±0.23	
A:G ratio	1.18±0.03	1.29±0.06	1.23±0.14	1.23±0.08	
Cholesterol (mg/dl)	118±21.16	131±19.10	126±16.30	130±18.86	

[1]The values in a row in italics are statistically different from each other (P≤0.05). Hb-Haemoglobin; PCV-Packed cell volume; ALT-Alanine amino transferase; SGPT-serum glutamic pyruvic transaminase; AST-Aspartate amino transferase; SGOT-Serum glutamic pyruvic transaminase and A:G ratio-Albumen:Globulin ratio

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Table 5. Haematological parameters of T3 age group birds

Parameters	Control	Treatments			Significant
		TA3	TB3	TC3	
Hb (g/dl)*	8.1±0.24[a]	9.2±0.19[b]	9.55±0.62[b]	10.48±0.49[c]	Improvement in general health condition of the birds enhanced the value of Hb and PCV and glucose
PCV (%)*	26±6.25[a]	32±3.89[b]	32±3.86[b]	34±2.38[b]	
Glucose (mg/dl)	129±12.05[a]	139±21.51[ab]	146±21.10[ab]	161±19.54[b]	
ALT (U/L)/SGPT	3.85±0.11	4.08±0.65	4.22±0.66	4.71±0.59	
AST (u/L) /SGOT	149±14.02	151±12.29	155±21.20	160±21.16	
Creatinine (mg/dl)	1.56±0.15	1.38±0.24	1.46±0.34	1.16±0.34	
Total protein (g/dl)*	4.18±0.26[a]	4.48±0.16[ab]	4.52±0.31[ab]	4.88±0.25[b]	No drowsiness and off feed conditions and improvement in nutritional status of birds
Albumen (g/dl)	1.89±0.19	1.98±0.21	1.95±0.26	1.98±0.13	
Globulin (g/dl)	2.29±0.31	2.5±0.25	2.57±0.21	2.9±0.16	
A:G ratio	1.21±0.07	1.26±0.11	1.32±0.09	1.46±0.08	
Cholesterol (mg/dl)	95±10.21	109±14.51	116±13.31	122±16.90	

[1]The values in a row in italics are statistically different from each other (P≤0.05). Hb-Haemoglobin; PCV-Packed cell volume; ALT-Alanine amino transferase; SGPT-serum glutamic pyruvic transaminase; AST-Aspartate amino transferase; SGOT-Serum glutamic pyruvic transaminase and A:G ratio-Albumen:Globulin ratio

CONCLUSION

The results of the present experiment indicate that the new herbal drug "Actovet-CRD" could be effectively used against the CRD complex as well as other respiratory distress syndromes in the poultry in most natural way. A.CRD improved the body weight gain, survivability, haemoglobin, PCV, total protein levels to a significant level. The results indicated that the birds of age group T1, T2 and T3 should be supplemented with A.CRD at the rate of 10, 20 and 40 ml/100 birds respectively.

In the modern world where we are in search of organic agriculture, organic animal husbandry etc. this drug can be a solution to organic poultry farming. The farmers were satisfied with the efficacy of this drug in treating the CRD. Few farmers also have obtained higher price as the products are sold under organic poultry farming products.

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