IMPACT-P(NA)

"The Litter Vaccine"

IMPACT
poultry products
By Environmental Dynamics, Inc.

Effectiveness in Reducing "Gram Negative" Disease Causing Bacteria and Ammonia in Poultry Litter

In both field trials and scientific studies, IMPACT-P(NA) has proven to be an effective, non-toxic, litter amendment that competitively excludes disease causing bacteria and reduces ammonia during growout.

One illustrative study[1] raised broilers for 49 days in eight (8) pens with litter treated with IMPACT-P(NA) and eight (8) pens with no litter treatment (the control). At the end of growout the researchers found that those pens treated with IMPACT-P(NA) had significantly lower ammonia than the controls, lower populations of potentially disease causing "Gram Negative" bacteria in the litter, as well as lower populations of disease causing "Gram Negative" bacteria on the bodies of the broilers.

IMPACT-P(NA) includes artificially high populations of beneficial, "Gram Positive," *Bacillus* bacteria that out compete other bacteria for nutrients and secrete compounds that inhibit the growth of bacteria that commonly populate poultry litter. In addition, the bacteria in IMPACT-P(NA) composts litter in place and facilitates the transformation of ammonia to stable nitrogen forms of nitrite and nitrate. Lower populations of disease-causing bacteria, lower ammonia levels and healthier litter results in healthier birds and better settlements flock after flock.

Litter Ammonia

Mean ammonia reduction in IMPACT-P(NA) treated litter was 50%. This is consistent with air sampling test results collected over several years in actual growout conditions.

Litter Ammonia		
Pen	**Control (ppm)**	**Treated with IMPACT P-(NA) (ppm)**
1	15	10
2	50	5
3	35	40
4	30	25
5	20	10
6	55	10
7	25	5
8	35	30
Avg.	**33.13**	**16.88**
St. Dev.	12.98	12.23

Litter Bacteria

Populations of potentially disease causing "Gram Negative" bacteria (like E.coli, Campylobacter, and Salmonella) were more than 50% lower in litter treated with IMPACT-P(NA).

"Gram Negative" Bacteria in Litter		
Pen	**Control (# per ml)**	**Treated with IMPACT P-(NA) (# per ml)**
1	246	102
2	154	85
3	302	137
4	214	196
5	139	165
6	441	43
7	152	87
8	207	49
Avg.	**231.88**	**108.00**
St. Dev.	94.11	50.68

Whole Bird Exterior Bacteria

Populations of potentially disease causing "Gram Negative" bacteria (like E.coli, Campylobacter, and Salmonella) were more than 50% lower on the exterior of birds grown on litter treated with IMPACT-P(NA).

"Gram Negative" Bacteria on Whole Bird Exterior		
Pen	**Control (# per ml)**	**Treated with IMPACT P-(NA) (# per ml)**
1	69	15
2	16	6
3	54	33
4	38	49
5	48	15
6	93	8
7	32	29
8	57	18
Avg.	**50.58**	**21.63**
St. Dev.	22.07	13.53

1. Parc Institute (1999). *Evaluation of IMPACT-P(NA) effectiveness in reducing pathogens and ammonia in litter.*

Need more info? Please contact us at **800-448-4723** or **info@impactpoultryproducts.com**.

Use of IMPACT Program to Deter Necrotic Dermatitis

Northwest Arkansas producer's experience: 3 houses, one with mild dermatitis break other 2 without problem. Neighboring farm with 5 houses, experienced severe dermatitis in one house and less severely in 2 other houses. Both growers were in the same settlement week for the same Integrator.

Grower A had implemented the IMPACT Program as remedy for poor performance and recurring dermatitis problems 3 flocks previously. The complete IMPACT Program consisted of thorough litter removal and washdown followed by deep penetration floor cleansing with the IMPACT-S process. New litter was seeded with IMPACT-P(NA) at recommended rate of application, 1 lb per 1,000 square feet.[1] The flock settled #2 with no evidence of dermatitis. IMPACT-P(NA) was used for each subsequent flock as prescribed. On day 35 of the third flock post PROGRAM treatment dermatitis broke in one of the three houses.

Farm A time line:

Day 35 (first broke)	10 dead
Day 36	40 dead
Day 37	70-75 dead
Day 38	70-75 dead, Spraying IMPACT was recommended. Integrator started combination treatment with Propionic acid 65%, stock 1 gal to 4 gal water and lincomycin 80 grams in 2 gal water stock. Metered at 1 oz per gallon water through medicator. Continued for 2 days.
Day 39	Sprayed 1/2 of house with 5 pounds of IMPACT-P(NA). Continued medication with propionic acid / lincomycin.
Day 40	15 dead. No further medication used.
Day 41	15 dead.
Day 42 – 47	Consistent 10 – 20 dead per day. Flock out at 48 days

Neighboring farm B growing for same integrator and on same placement schedule broke with dermatitis on day 34 and was losing upwards of 250 birds daily. This farm also went onto a regimen of propionic acid & lincomycin. Medication was administered for 5 days on this farm. The integrator also applied 800 pounds of PLT (Jones Hamilton) to each of the affected houses. There was a drop from 245 to 145 dead for one day after beginning the medication regimen and then mortality again increased to the 250 per day level. The farm went onto Penicillin at day 41 using $1200 worth over a 4 day period to control the dermatitis.

Timeline on farm B. 5 houses, one with severe break 2 others less severe dermatitis

Day 34	First noticed dermatitis. Lost 50 birds and had been picking up 20-30 per day.
Day 35	100 dead
Day 36	245 dead, Started on propionic acid / lincomycin medication for 5 days
Day 37	145 dead, Integrator applied 800 lbs. PLT
Day 38	250 dead
Day 39	279 dead
Day 40	250 dead
Day 41	250 dead, Flock started on penicillin
Day 42 - 44	Continued on penicillin and losing 40-50 birds/day.
Day 45	Restarted on propionic acid / lincomycin and continuing to lose 40-50 per day.

It is notable that even though dermatitis did break in the IMPACT treated house, the disease was less severe than in the neighboring untreated house(s). IMPACT-P(NA) claims to improve the growing environment by shifting microbial populations in favor of the beneficial waste degrading organisms it contains. The resulting competitive exclusion and competitive inhibition lessens the bacterial stress and healthier birds are less susceptible to disease processes.[2] This field situation provided an excellent opportunity to test the value of adding additional IMPACT organisms in an effort to actually retard the progression of a dermatitis outbreak. The difference between the farms, A & B is the history of IMPACT use on the litter in Farm A and the "shock" application of IMPACT at the first sign of a dermatitis outbreak. The combination of medication and IMPACT showed retardation of the normal progression of dermatitis infection. Also notable is the apparent ineffectiveness of medication and PLT in controlling the progression of dermatitis on farm B.

Cost assessment:

Farm A			Farm B	
10 lbs IMPACT-P(NA)	$95.00		800 lbs PLT	> $160.00
2 days antibiotics	Integrator Expense		5 days antibiotics	Integrator Expense
Excess mortality	120 -150 birds		120 paks Penicillin	> $1,200.00
			Excess Mortality	1100 – 1200 birds

Need more info? Please contact us at **800-448-4723** or **info@impactpoultryproducts.com**.

[1] IMPACT Program applied by 4-States Poultry, Springdale, AR

[2] "Ecological Intervention with IMPACT-P(NA)", Environmental Dynamics, Inc. Buena Vista, VA

EFFECT OF USING IMPACT-P(NA) ON LITTER IN BROILER AND BREEDER FARMS HAVING SALMONELLA

Case 1: 49 broiler flocks with history of *Salmonella enteritidis* and reported positive by processing center were chosen for use of IMPACT-P(NA). The flock received IMPACT-P(NA) on the litter (the same litter, without any other treatment). All of them were negative for *SE* at time of processing.

Grower	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Previous flock	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IMPACT-P(NA) flock	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Grower	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Previous flock	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IMPACT-P(NA) flock	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Grower	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Previous flock	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IMPACT-P(NA) flock	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Case 2: 2 breeder flocks with a history of *Salmonella* were detected positive for *SE*: the first at the 23rd week and the second at week 50. Both were treated with enrofloxacin for 10 days and received IMPACT-P(NA) on the litter. *SE* were not detected on the litter in following weeks, and the flocks remained negative for *SE*.

Flock 1															
Age (weeks)	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Salmonella enteritidis	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Flock 2														
Age (weeks)	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Salmonella enteritidis	-	+	+	-	-	-	-	-	-	-	-	-	-	-

Several other flocks were treated with enrofloxacin but not followed with IMPACT-P(NA), and that all flocks treated in this manner reverted to positive testing within three weeks after being treated with enrofloxacin.

COMBATING DERMATITIS WITHOUT EXPENSIVE ANTIBIOTICS

Very high populations of IMPACT bacteria applied at one time **(IMPACT-P)**[3] have been demonstrated to help improve deteriorating performance on built up litter (Perdue 2005; Jordan Farm, Patrick, SC 2.8% improved weight gain and 4 points better Feed Conversion); overcome aspergillus problems (Perdue, Western NC Breeder operations).

Farm A / IMPACT-P		Farm B / no IMPACT-P
Broke on day 35		Broke day 34, 50 dead
Day 36-39 losses: 40 - 70 - 75 /day		Day 35 - 36 losses: 100 - 245
Day 38 Started propionic acid/ lincomycin for 2 days		Day 36 sodium bisulfate applied and started propionic acid / lincomycin for 5 days.
Day 39 sprayed½ with IMPACT		Day 37 lost 145
Day 40 sprayed ½ with IMP ACT		Day 38- 41 losses: 250, 279, 250
Day 40 lost 15 birds		Day 41 started on penicillin
Day 41 lost 15 birds		Days 42-44 losses: 40-50/day
Days 42 - 48 lost IO - 20/day		Day 45 restarted Propionic acid/lincomycin and continued to lose 40-50/day.

Cost for Farm A: less than $300.00 plus the excess mortality of 120 - 150 birds and integrator's cost for propionic acid and lincomycin.

Cost for Farm B: in excess of $2500.00 penicillin expense plus the excess mortality of 1100 - 1200 birds, and the integrator's cost for 800 lbs. PLT and propionic acid / lincomycin. [4]

EFFECTS OF USE OF IMPACT-P ON CELLULITES ON BROILER CHICKENS

Research in Brazil done at Institute of Veterinarian Researches Desiderio Finamor, supported by Schering-Plough concluded: "According to the results obtained through this work, we could conclude that: The use of five grams of IMPACT-P for each square meter of chicken litter **reduced the pathogenic *Eschericia coli* population to levels unable to cause avian cellulitis**." [5] See attached report.

IMPROVED GROWOUT PERFORMANCE

	weight gain	Feed Conversion	Livability
Average	**1.92%**	**3.96 points**	**1.93%**
Median	**1.2%**	**3 points**	**0.8%**

Statistics from 54 different field trials, 14 Integrators & several complexes [6]

2 successive growout trial by Virginia Integrator using IMPACT-P(NA)® on one house for first trial and other house for second trial. In both trials the IMP ACT treated house outperformed the untreated house. [7]

(2 flock totals)	Net lbs produced	pay per lb.	settlement
IMP ACT-P(NA)® treated	292,305	0.04435	$12,963.73
Untreated	283,732	0.03580	$10,157.61
Variance	8,573	0.00855	$2,806.12

These houses were cleaned out after each growout before placing new flocks.

- The grower realized $6.07 return for $1.00 cost of product
- The Integrator realized an average cost to produce savings of $0.01225 per lb.

FLOCK EVALUATION PROGRAM BY CENTRAL VIRGINIA GROWERS

The average weight delivered from a house per the Integrator was 120,000 lbs. That multiplied by the average gain in DFM (Difference From Middle) in cost per lb to produce provided the gain in dollars for the 3 flocks.

Formula Ex: 6 flock DFM less the 3 test flock average DFM X 120,00 lbs = $ benefit
Using farm 2 -.31 less +.12 = +.43 ($.0043) X 120,000 lbs = $516.00
S516 is the average gain/flock/house
S516.00 less $165 P{NA) cost = *$* 351.00 net profit gain/flock/house (before rebate)

Farm	Start Dates	Sell Dates	DFM per flock	6 flock DFM	Gain$	IMPACT cost$	Net$ gain
I	12/7/09	1/11/10	0.24	0.09	180.00	(495.00)	
	2/11/10	3/8/10	0.20		132.00		
	5/3/10	6/8/10	0.02		(84.00)		
Average			**+0.15**	**+0.06**	**+232.00**		**(263.00)**
2	12/24/09	1/29/10	0.29	(0.31)	720.00	(495.00)	
	2/18/10	3/26/10	0.08		468.00		
	5/14/10	5/14/10	0.00		372.00		
Average			**+0.12**	**+0.43**	**+1,560.00**		**+1,065.00**
3	12/7/09	1/11/10	0.90	0.17	876.00	(495.00)	
	1/27/10	3/3/10	0.55		456.00		
	3/9/10	4/23/10	0.38		252.00		
Average			**+0.61**	**+0.44**	**+1,584.00**		**+l,089.00**
4	3/5/10	4/9/10	0.62	0.10	624.00	(495.00)	
	4/23/10	5/28/10	0.12		24.00		
	6/9/10	7/16/10	0.63		636.00		
Average			**+0.46**	**+0.36**	**+l,284.00**		**+789.00**
5	1/14/10	2/18/10	0.47	(0.14)	732.00	(495.00)	
	3/8/10	4/14/10	0.46		720.00		
	4/23/10	5/31/10	0.63		924.00		
Average			**+0.52**	**+0.66**	**+2,376.00**		**+l,881.00**
6	1/19/10	2/23/10	0.47	(0.12)	708.00	(495.00)	
	3/12/10	4/17/10~	0.02		168.00		
	4/29/10	6/24/10	0.03		348.00		
Average			**+0.17**	**+0.29**	**+1,224.00**		**+729.00**
7	12/15/09	1/22/10	0.59	0.00	708.00	(495.00)	
	2/8/10	3/15/10	(0.31)		(392.00)		
	3/29/10	5/5/10	0.22		264.00		
Average			**+0.17**	**+0.17**	**+580.00**		**+85.00**
8	12/21/09	1/25/10	0.25	(0.17)	504.00	(495.00)	
	2/12/10	3/19/10	0.28		540.00		
	4/2/10	517/10	0.59		912.00		
Average			**+0.37**	**+0.54**	**+1,956.00**		**+1.461.00**

FULL COMPLEX SCALE TRIAL

Virginia Integrator qualified the IMPACT-P(NA)® program using contract service application over 67 settlements comparing performance to on farm controls, weekly average performance and 7 flock history of house performance. [8] The following quote is taken from the letter sent by the broiler manager to all growers.

"The 67 flocks, treated with IMPACT-P, show an improvement of .34 cents per live pound compared to the 7-flock ICF for the same houses. The 22 test flocks show a relative advantage of .39 cents per live pound over the 22 control flocks and an improvement of .39 cents per live pound compared to the 7-flock ICF for the same houses."

This same Integrator in 2002 reviewed 19 growers consistently using IMP ACT-P(NA) for the past year and compared them to the overall company performance, The benefit was .33 cents per live pound better than the company average and a 4 house farm yielded greater than $6,000.00 net improvement over the entire year's use of IMPACT-P(NA). [9]

Roaster growout evaluation run by large Delmarva Integrator to measure comparative results of IMPACT-P(NA)® versus control and versus PLT. [10]

parameter	PLT	IMP ACT-P(NA)®	Control
roaster livability	94%	96%	95%
average weight	7.89 lbs	8.25 lbs	7.88 lbs
cost to produce	0.2423/lb	0.2416/lb	0.2454/lb

Natural bird producer, Clark's Feed Mill, Shamokin, PA began using IMPACT-P(NA)® in 1996 and saw its success rate in raising chickens without antibiotics climb to in excess of80%[11]

CLEANER BIRDS FROM A CLEANER ENVIRONMENT

Statistics from a large trial conducted by a Virginia Integrator where more than 500 settlements to date have been treated with IMPACT-P(NA)®[12]

Indications of overall flock health in trial sets:

• Condemnation:	• **33% lower**
• Incidence of air saculitis:	• **57% lower**
• Improvement in Grade A paws:	• **5% increase**

BIOLOGICALLY STABILIZED LITTER NUTRIENTS
Proactive Litter Waste Management

The IMPACT-P(NA)® formula contains ingredients to help facilitate composting of litter waste. This composting action improves litter quality by:

- ⇒ stabilizing Phosphorous and Nitrogen
- ⇒ reducing the off-gassing of Nitrogen in the form of ammonia for an entire growout
- ⇒ conditions litter for re-use.

Litter samples from 26 houses grown for one flock were analyzed by Ag Consulting Lab and NC Dept. Ag for nitrogen and phosphorous content. 8 single flock litter samples treated with IMPACT-P(NA)® were also analyzed by NC Dept. Ag. [13]

	26 control	8 IMPACT-P(NA)®	Variation%
Phosphorous ppm	15,804	14,310	**-9.5%**
Nitrogen ppm	44,340	41,844	**-5.6%**

A 4 house and 2 house farm under same management in Siler City, NC were tested for nutrients after the second flock and 3rd flock on the litter.[14] The phosphorous data shows that there was less measureable soluble phosphorous accumulation in IMPACT-P(NA)® treated houses than in untreated houses by 5.8% in houses treated with standard rate of 1 lb per 1,000 square feet. The other farm (PLF) used 1.5 lbs per 1,000 square feet and the data shows an actual overall reduction in phosphorous of 0.8%.

Houses	Ave P before	Ave P after	change	%
BLF #1&2 control	14,961	17,875	+2,914	+ 19.5%
BLF #3&4 treated	16,321	18,561	+2,240	+ 13.7%
PLF #1 control	14,362	17,974	+3,612	+25.1%
PLF #2 treated 1.5X	15,561	15,428	-133	-0.8%

In 1992 litter from a Cameron, NC trial[15] was analyzed for 3 consecutive flocks on old litter. The Nitrogen was analyzed for TKN and NO_3-N. The data shows stabilization as the NO_3-N fraction increases with successive flocks and the ratio of TKN to NO_3-N shifts in favor of the stable nitrogen form, NO_3-N.

Litter Batch #2 Flock#3 on litter 7-16-92

Parameter	Control	**Treated**	Variance	%
Phosphorous	2418.23	**1930.20**	-488.03	-20.18%
NO_3-N	125.3	**125.97**	0.67	0.5%
TKN	16,238.67	**16,912.00**	673.33	4.1%
TKN / NO_3-N ratio	129.6 / 1	**134.2/1**		

Flock #4 on litter 9-10-92

Parameter	Control	**Treated**	Variance	%
Phosphorous	1,928.5	**1,570.67**	- 257.83	-18.5%
NOrN	222.56	**174.13**	- 48.43	-21.8%
TKN	14,242	**13,125.67**	- 1,116.33	-7.8%
TKN / NO_3-N ratio	64/ 1	**75.4 / 1**		

Flock #5 on litter 1-12-93

Parameter	Control	**Treated**	Variance	%
Phosphorous	3,439	**3,344**	- 95	-2.8%
NO_3-N	194.4	**204.2**	+9.8	+5.0%
TKN	17,343	**13,917**	- 3,432	- 19.75%
TKN / NO_3-N ratio	89.2 /1	**68.15 /1**		

IMPACT'S "ECOLOGICAL INTERVENTION" CONDITIONS LITTER

In April, 1996 ammonia readings were taken in the litter of broiler houses that had been treated with IMPACT-P for 5 consecutive growouts on the same litter. Readings were taken from 2 control houses on the same farm. [16]

House	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Test #l	10	10<15	10<15	10	5<10	10
Test #2	10<15	10	10<15	10	5<10	10
Control #1	>100	>100	>100	>100	90>100	>100
Control #2	>100	>100	>100	>100	>100	>100

All measurements by GasTech draw tube in ppm

[1] Evaluation of IMPACT-P(NA) effect on Salmonella enteritidis. Oct. 2000 reported by Coopers Brasil Ltda, Div. Schering-Plough, conducted at Frangosul.
[2] Evaluation of IMPACT-P(NA) effects on litter bacteria. PARC Institute, Easton, MD, August 1999.
[3] IMPACT-P is a high population blend of IMPACT-P(NA) microbes that is activated at one time for extremely high contribution of waste degrading IMPACT bacteria to poultry litter.
[4] NW Arkansas, 2003, Evaluation of cost and effectiveness in fighting concurrent dermatitis outbreaks with conventional antibiotics and IMPACT Products.
[5] Effect of the use of IMPACT-P on the occurrence of cellulites in broiler chickens. Benito Guimaraes de Brito, et.al, Institute of Veterinarian Researches Desiderio Finamor, Eldorado do Sul - RS Brazil 2006.
[6] Conducted between 1992 - 1995 using IMPACT-P. Tests encompassed all seasons, normal variations in bird quality, feed quality and management practice. Results were achieved in hot weather with open houses and cold weather with closed and heated houses.
[7] Field Evaluation of IMPACT-P(NA), Harrisonburg, VA March - June 1996.
[8] Complex level evaluation of IMPACT-P(NA) effects on broiler production. Harrisonburg, VA 1996.
[9] 2001-2002 George's Poultry review of IMPACT-P(NA) use versus company performance average.
[10] Cookin Good Farm trial, 1997.
[11] Clark Feed Mill testimonial letter, Jan. 1997.
[12] Complex level evaluation of IMPACT-P(NA) effects on broiler production. Harrisonburg, VA 1996
[13] Rocco Farms litter report, Oct. 1998
[14] Evaluation of litter Phosphorous and Nitrogen accumulations in old litter treated with IMP ACT-P(NA). Siler City, NC, Sept. - Dec. 1998
[15] The Effects of IMPACT-P Bacterial Treatment of Poultry Litter, Analysis for Nitrogen and Phosphorous. Golden Poultry, Cameron, NC 1992
[16] Chas. Hill Poultry Farm, Lineville, AL. Effects of IMPACT-P Treatment on Poultry House Litter.

Effect of the use of Impact-P® on the occurrence of cellulites on broiler chickens

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ABSTRACT

Necrotic dermatitis, popularly known as avian cellulitis stands out as the cause of the disposal of carcasses in abattoirs all over the world. The manifestation of this infirmity is related to several factors including the conditions of production such as nutrition, management, health and environment. *Escherichia coli* has been the bacteria most commonly associated to cellulitis and it is present in high quantity in broiler litters of aviaries. This study was designed to evaluate the effect of IMPACT – P® on the *Escherichia coli* population reduction and its result on the control of avian cellulitis. On the first experiment we used *Escherichia coli* plate counts on the times 0, 24, 48 and 72 hours after the treatment on the litters. On the second experiment, we evaluated the score of cellulitis lesions on inoculated broiler chickens with litters treated with IMPACT – P® and non-treated litters. The summing of IMPACT – P® was capable to extensively reduce the amount of *Escherichia coli* on the chicken litters after 24 hours of contact. The effect also prevented in a significant way the occurrence of cellulitis in chickens exposed to pathogenic *Escherichia coli.*

INTRODUCTION

Brazil took the worldwide leadership in chicken meat exportation incomes in 2003. These results, however, don't depend only on the achieved productivity, but on the quality of the final product. All broilers production converges to the abattoirs where the sanitary inspection service examines them. The result of these examinations defines the destination of the carcass, which can be fully or partially liberated or discarded. During this intermediary stage, between production and sales, the expected income is severely reduced due to the alterations detected on the carcasses.

In the last few years, the occurrence of integument problems has been increasing in the abattoirs. Many of these skin lesions are generically classified as "dermatosis", due to the difficulty to determinate the pathology of the problem. Necrotic dermatitis, popularly known as avian cellulitis stands out as a cause of the carcasses disposal in abattoirs all over the world. The problem will only be detected during the inspection at the slaughter. It occurs even in birds coming from flocks with a good performance. The manifestation of this infirmity is related to several factors including the conditions of production such as nutrition, management, health and environment. *Escherichia coli* has been the microorganism most commonly associated to cellulitis. However, other pathogens may be associated to the lesions manifestation. Currently, this infirmity is responsible for nearly 30% of the disposals, in countries with high production rates. In Brazil, cellulitis is an increasing cause of carcasses disposal, resulting in a loss superior to 10 million dollars.

According to Elfadil et al. (1996) the cellulitis lesion is caused by many factors and the presence of certain risk factors predisposes its occurrence. Among the risk factors related to the problem we list the size of the farm, abdominal lesions, dermatitis and reutilization of the litter.

The plan of biosafety in aviary productions, among other factors, alleges the maintenance of an environment with low incidence or free of microorganisms that may interfere in the production. This would avoid the incoming of pathogenic agents that may disturb the sanity, well being and performance of the birds. (JAENISCH, 1999) The reutilization of the chickens litter after it being cleaned up is an alternative that supplies the needs in aviculture such as the maintenance of the farm and consumer's health, and also the reduction of the environment impact and additional costs. The combination of the present factors on aviary litters such as pH, temperature, organic compounds and water activity, allows the bacteria development, especially aerobic mesophilic or microaerophilic. The bacterial inactivation in aviary houses can be done in many ways, such as acidification, fermentation, liming and reduction of the water activity. These methods must be followed by an evaluation of their efficiency, since their success may vary in different situations. (FIORENTIN, 2005) IMPACT – P® is an indicated product for this purpose, since it's biochemically active and developed from *Bacillus subtilis* and its enzymes (proteases) which act on animal wastes and organic matter, reutilizing them as food. As a result, the levels of ammonia tend to be reduced, improving the environment general conditions in the ambient where the birds are created. The product acts on the nitrogen cycle, easing the conversion of the ammonia into nitrate and nitrite (SCHERING-PLOUGH, 2006).

This experiment was executed to evaluate the control of avian cellulitis, through the utilization of IMPACT – P® in broilers litter.

MATERIAL AND METHODS

The experiments were performed in the Instituto de Pesquisas Veterinárias Desidério Finamor (Institute of Veterinarian Researches Desidério Finamor) – FEPAGRO, in Eldorado do Sul, state of Rio Grande do Sul, Brazil. The methodologies regarding Biosafety are certified by the register in the Internal Biosafety Commission – CIB 001/06 and CIB 002/06.

The litters used in the experiments were made of woodshaving already used in a creation of broiler.

For the first experiment we studied the reduction of the *Escherichia coli* population in broilers litters after the IMPACT – P® treatment. Two treatments were performed in this experiment, as described below:

T1: Litter treated with IMPACT - P® (5 g/m^2);

T2: Control, litter without IMPACT - P®.

IMPACT – P® contains 3,3 x 10^8 UFC/g of *Bacillus subtilis*. The dose used for this experiment in the litter was 5g/m^2 of surface, administered directly over the wood shavings.

The inoculum was produced from the *Escherichia coli* sample, originator of cellulites in birds (Ecolvet 1), carrier of the virulence genes (F11, *tsh*, *iut*A, *col*V, *tra*T, *kps* and *fim*H) (BRITO et al., 2003). The sample was cultivated in *tryptic soy broth* for 24 hours in a temperature of 98.6 °F. This bacterial sample was inoculated in a wood shaving litter to reach a 1.0 x 10^7 UFC/g wood shaving concentration.

The population quantification of *Escherichia coli* was determined in the times 0, 24, 48 and 72 hours after the bacterial inoculation in the litter. The count was performed through the MacConkey Agar plate count method with incubation at 98.6 °F for 24 hours.

For the second experiment we evaluated the action of IMPACT – P® on the occurrence of avian cellulitis. We used 12 Cobb broiler chickens, 21 days old, separated in two groups comprising the treatments performed in this experiment, as described below:

T1: Animals inoculated with 0.1 mL of litter treated with IMPACT - P® (5g/m^2);

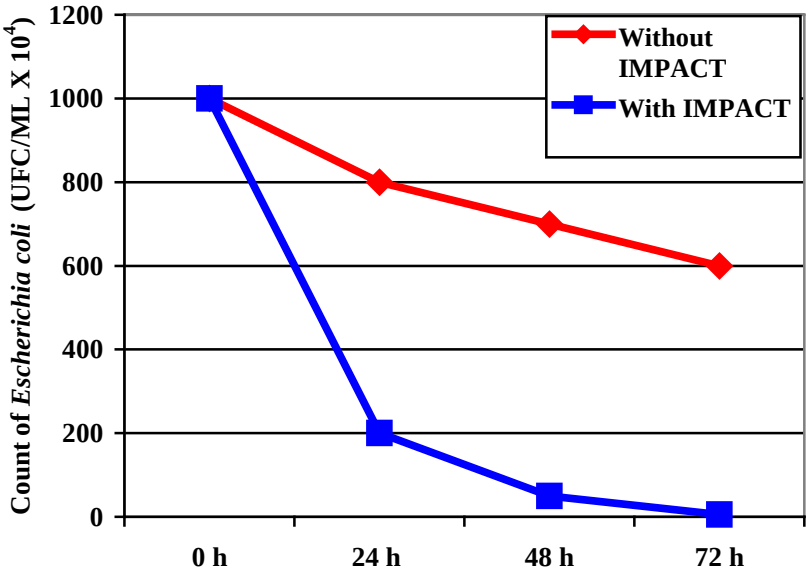
T2: Control, animals inoculated with 0.1 mL of litter without IMPACT - P®.

The inoculum was produced from the *Escherichia coli* sample, originator of cellulites in birds (Ecolvet 1), carrier of the virulence genes (F11, *tsh*, *iut*A, *col*V, *tra*T, *kps* and *fim*H). The sample was cultivated in *tryptic soy broth* for 24 hours in a temperature of 98.6 °F. This bacterial sample was inoculated in a wood shaving litter to reach a 1.0 x 10^7 UFC/g wood shaving concentration. In both treatments the litters kept in contact with the microorganism for 72 hours. The birds were inoculated with a 0.1 mL of a 1:10 solution of litter in saline buffer, via subcutaneous in the chest area.

To determinate the efficiency of IMPACT – P® we performed evaluations on the chickens in the inoculations spots after 24 hours. The birds were sacrificed, autopsied and examined to verify the presence of cellulitis lesions. From the inoculation local we collected skin and muscle fragments to re-isolate it from the microorganism. The cellulitis lesions were classified from 0 to 4, being score 0 = absence of visible lesion; 1 = slight opacity and thickening of the skin with fibrinopurulent exudation in the subcutaneous tissue; 2 = fibrinocaseous exudation smaller than 1 cm; 3 = fibrinocaseous exudation larger than 1cm; 4 = similar to score 3 with petechial hemorrhages and ecmosis in the muscles and skin (Brito et al., 2003). The test results of challenge in chickens were analyzes through Fisher test, with significance level of P≤0,05, with the statistic program Epi Info, Centers for Diseases Control and Prevention, Atlanta, Geórgia (EPI INFO, 1994).

RESULTS AND DISCUSSION

In the first experiment related to the *Escherichia coli* count in chicken litters treated and non-treated with IMPACT – P®, we observed a reduction in this microorganism population in all evaluated times with the use of the product (Graphic 1). This reduction is accentuated on the third day of contact with IMPACT – P®, showing that this bacterial population reached a level which it's incapable of causing damage to the birds health. Epidemiologic studies, using molecular techniques, have suggested that certain clones of *Escherichia coli* may be specific for cellulitis. (NGELEKA et al., 1996). The *Escherichia coli* samples isolated from cellulitis in broiler chickens are endemic in aviaries and poultry farms (SINGER et al., 2000).



Graphic 1. *Escherichia coli* count in chicken litters treated and non-treated with IMPACT – P® (5g/m^2).

In the second experiment we could observe that birds inoculated with litter without IMPACT – P® suffered a reproduction of score 4 cellulitis, with fibrinocaseous exudation larger than 1cm, petechial hemorrhage and ecmosis in the muscles and skin (Figure 1), with re-isolation of the microorganism. Birds inoculated with litter treated with IMPACT – P® didn't suffer with the formation of this inflammatory process (Figure 2) or with re-isolation of the microorganism. We could classify them with score 0, and consider this difference highly expressive (P<0,05). These results show that IMPACT – P® was significantly capable of reducing the occurrence of cellulitis in chickens due to the reducing of the *Escherichia coli* population, the causer of avian cellulitis.

Figure 1. Broiler chicken inoculated with 0.1 mL litter treated with *Escherichia coli* without IMPACT – P®, score 4 in cellulitis lesion.

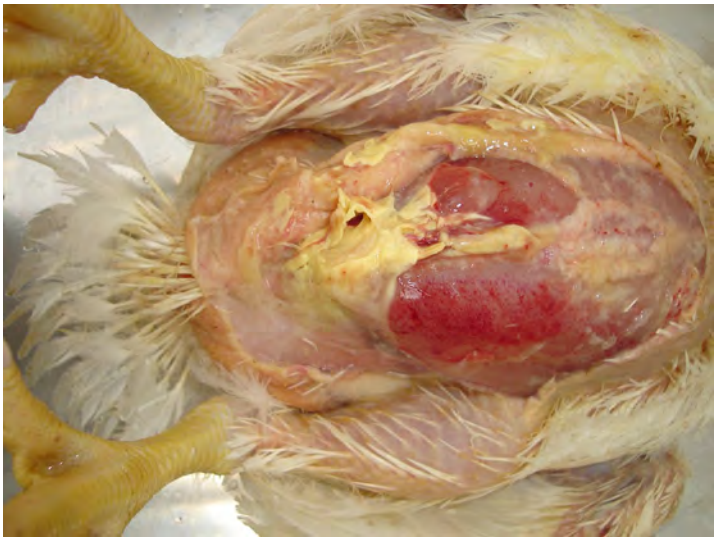
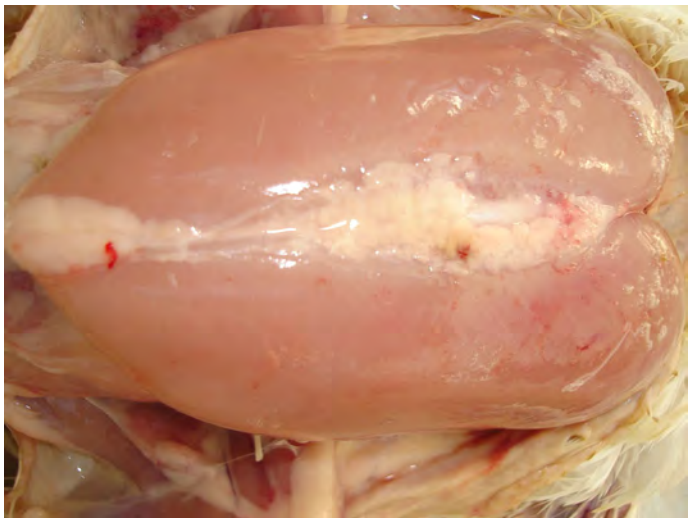


Figure 2. Broiler chicken inoculated with 0.1 mL litter treated with *Escherichia coli* with IMPACT – P®, score 0 in cellulitis lesion.



Avian colibacillosis is an infirmity of great economic impact and it's caused by certain virulent samples of *Escherichia coli* (BRITO et al., 2003). There are many forms of colibacillosis which could be mentioned: Chronic respiratory disease, omphalitis, septicaemias, diarrheas and cellulitis (BARNES & GROSS, 1997). The control of cellulitis, which is the cutaneous form of this disease, has been a challenge for the researches. It is known that this infirmity is caused by many factors and the measures to control it haven't showed a good practical result so far. Recently, natural measures of control have been proposed, since the conventional antimicrobial alternatives have had their use restricted. Our results agree with these facts, since the product contains microorganisms of low pathogenicity to animals and expressive interaction in the reduction of pathogenic *Escherichia coli* in chicken litters, and consequent control of the avian cellulitis.

CONCLUSIONS

According to the results obtained through this work, we could conclude that:

The use of five grams of IMPACT – P® for each square meter of chicken litter reduced the pathogenic *Escherichia coli* population to levels unable to cause avian cellulitis.

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BIBLIOGRAPHIC REFERENCES

BARNES, H. J.; GROSS, W. B. Colibacillosis. In: CALNEK, B. W.(Ed.) **Diseases of Poultry.** 1997. p. 131-141.

BRITO, B.G.; GAZIRI, J.; VIDOTTO M.C. Clonal relationships among strains and virulence factors of avian *Eschericha coli* from cellulitis aviária. **Infection Immunity,** v.71, p.4175-4177, 2003.

ELFADIL, A.A.; VAILLANCOURT, J.P.; MEEK, A.H. Farm management risk factors associated with cellulites in broiler chicken in southern Ontario. **Avian Diseases,** v.40, p699-706, 1996.

EPI INFO (1994). version 6.02: a world processing, database and statistics program for epidemiology on microcomputers. Atlanta, GA: **Centers for Disease Control and Prevention**.

FIORENTIN, L. Implicações da reutilização da cama de aviário para a saúde pública e animal. In: IV SEMINÁRIO INTERNACIONAL DE AVES E SUÍNOS, 2005, Florianópolis. **Anais...** Florianópolis:AVESUI, 2005. p. 35-41.

JAENISCH, F. R. F. Biossegurança em plantéis de matrizes de corte. **Avicultura Industrial**, v. 90, n. 1072, p. 14-19, 1999.

NGELEKA, M.; KWAGA, J. K.; WHITE, D. G. *Escherichia coli* cellulitis in broiler chickens: clonal relationships among strains and analysis of virulence-associated factors isolates from disease birds. **Infection and Immunity**, v. 64, p. 3118-3126, 1996.

SCHERING-PLOUGH. Produto – IMPACT- P (NA). Available at: <http://www.spah.com.br/script/CprProd.asp?plntCodProduto=228>. Access in: July 18th, 2006.

SINGER, R. S.; JEFFREY, J. S.; CARPENTER, T. E. Persistence of cellulitis-associated *Escherichia coli* DNA fingerprints in successive broiler chicken flocks. **Veterinary Microbiology**, v. 75, p. 59-71, 2000.

Broiler breeder microbiological litter condition following treatment with Impact-P®

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ABSTRACT

This study aimed to evaluate the utilization of Impact-P® in used litter from floor pens of broiler breeders through the monitoring of microbiological parameters. Impact-P® is a commercial product obtained from ***Bacillus subtillis*** and it enzymes. Using animal litter and organic matter as a feed source, the product was designed to improve the quality of floor pens of broiler breeders. When hens aged 58 weeks, all used floor pens were replaced with a new one and the following treatments were tested: T1 - control; T2 - 2.5g Impact-P® m^{-2} litter; T3- 5.0g Impact-P® m^{-2} litter. Counting of number of enterobacterium colonies in Mac-ConcKey agar was performed in a sample of new material and after 3, 10, 17, 24 and 31 days of litter treatment with Impact-P® . The statistical analysis was performed with the General Linear Models - GLM procedure, using the minimum square method in a 3x4 factorial (treatment vs. time of floor pens utilization), in a completely randomized design with four replications. The averages were compared by Tukey test. The use of 5.0g Impact-P® m^{-2} litter was efficient to reduce approximately 13% logarithmic total counting of the enterobacterium in comparison to the untreated group (2.89 *versus* 3.31$\log_{10}$ (CFU), respectively, P <0.05).

Key words: litter, enterobacterium, gram negative bacteria.

This study was published in Portuguese in the Brazilian agriculture science journal *Ciência Rural*. The following study summary was translated from Portuguese to English using Google Translate.

The aviary bed is a cover that varies from 5 to 10cm thick arranged on the floor of the shed, which uses various materials, such as sawdust or pine wood, eucalyptus, hardwood, rice husk, sugar cane bagasse , corn cob or straw can be renewed at each production cycle or reused in up to six batches (Ávila et al., 1992; Oliveira et al., 2003).However, bed reuse in successive batches makes it difficult to disinfect the environment by changing the microbiological quality of the production system (WALTER, 2000). This factor may contribute to the prevalence of microorganisms in the environment, such as ***Salmonella*** spp. (CHERNAKI-LEFFER et al., 2002). For this reason, it is necessary to develop and implement products that reduce the contamination of animals and foods consumed by man. In this sense, several substances have been added in poultry litter trying to improve their microbiological quality, such as, calcined or extinguished lime which is $Ca(OH)_2$ calcium hydroxide obtained by the reaction of virgin lime with water (SINGH et al. , ***Bacillus subtillis*** (BRITO & et al., 2001), and the ***Bacillus subtilis*** (***Bacillus subtillis)*** TAGLIARI, 2007).

Impact-P® is a product formulated through ***Bacillus subtillis*** and its protease enzymes, which act on the animal and organic matter present in the aviary bed, using them as a nutritional source with the unfolding and use of these substrates, reducing, for example, the levels of ammonia, thereby improving the overall environmental conditions within the aviary.
BRITO & TAGLIARI, (2007) found that the addition of Impact - P ® in broilers reduced the amount of ***Escherichia coli*** in the bed from 24 hours of contact and also significantly prevented the occurrence of cellulite in chickens exposed to strains of ***Escherichia coli*** . Thus, the present study was carried out with the objective of evaluating the effect of the use of Impact - P ® applied in different dosages on the control of the pathogenic microbial activity.

The experiment was conducted in the Experimental Aviary of the Agrotechnical Complex Visconde da Graça - CAVG - UFPEL. A total of 180 broiler matrices with 58 weeks of age and 24 roosters of the same age were housed in 12 boxes (17 birds / box - 4.25 birds m^{-2}) with bed composed of wood particles produced by the processing and planing of lumber of ***Pinus elliottii*** in logging, with specific gravity of approximately 85 kg m^3 and average grain size of 24.0 mm. Each treatment had four replicates, each box was defined as an experimental unit. The experiment lasted four weeks, which corresponds to the mean time of bed change within the litter management in the shed of broiler chickens in the Agrotécnico Visconde da Graça - CAVG / UFPEL. The treatments applied to the experimental units were as follows: T1 - Control (without application of the product); T2 - application of 2,5 g $m^{-2}$of Impact-P® on the bed of birds; T3 - application of Impact-P® 5g m^{-2} on the bed of birds.

For the microbiological analysis five samples were collected. The first one at the moment of distribution of the new bed in the experimental units, the second three days after the application of Impact-P® , the third, fourth and fifth collections at 10, 17, 24 and 31 days, respectively. Individual aliquots (collected at five equidistant points - one meter - four of them located at each corner and the fifth at the center of boxing) were collected from each experimental unit corresponding to a treatment, which were packaged in a single sterile plastic package, thus composing a single sample of each treatment. From these samples, enterobacteria were counted on MacConkey agar. In this medium, leavening and non-fermenting colonies of lactose were differentiated. Samples were weighed, and subjected to decimal dilutions (up to 10^{-4}) in 0.85% saline, and then homogenized and inoculated into MacConkey agar plates. After 24h of incubation in a greenhouse with a temperature of 37 ° C, the counts and differentiations of the colonies were made in positive or negative lactose.

The statistical analysis was performed in the General Linear Models - GLM procedure using the least squares method, in a 3x4 factorial scheme (treatment x bed time) in a completely randomized design, with four replications per treatment.
The means were compared by the Tukey test (P <0.05) according to the model: $Y_{ijk} = \mu + A_i + ß_j + (Aß)_{ij} + E_{ijk}$, where: Y_{ijk} = response variable in the repetition k, level j of ß and level i of A; µ = general mean; A_i = effect of the factor Impact-P® at the level (i = 1,2, 3); $ß_j$ = effect of the bed time factor at the level (j = 1,2, 3, 4); $(Aß)_{ij}$ = effect of the interaction Aß at level i, j; E_{ijk} = Random error.

As can be seen in Table 1 , the results show that the application of Impact-P® to the bed 5.0 g m^{-2} dosage, recommended by the manufacturer, provided better microbiological quality due to the lower logarithmic count of enterobacteria, presenting a reduction of 12.9% compared to the control group (2.89 *versus* 3.31 $\log_{10}$ (CFU), respectively, P <0.05) and also a significant reduction of 12.8% in relation to the sub-dosage application of 2 , 5g m^{-2} of the product (2.89 *versus* 3.30 $\log_{10}$ (CFU), respectively, P <0.05). It is also observed that bed time has significantly affected the count of enterobacteria, that is, birds gradually incorporate a large amount of waste into bed increasing their microbiological population. The interaction between treatment factors and time of bed use was not significant, therefore, the effects of the factors can be studied separately.

These results suggest that application of the dose recommended by the manufacturer of Impact-P® provides sufficient amount of ***Bacillus subtilis*** in bed capable of inhibiting or controlling the growth of other bacteria. These microorganisms produce proteases, enzymes that break peptide bonds between the amino acids of proteins, which act on animal waste and organic matter from the aviary bed, degrading them. According to KIEHL, (2004) the nature of the microbial population, the number and species exist depend on the favorable conditions present in the substrate. Thus, the application of Impact-P® at the recommended dose provides an unfavorable environment for the growth of microorganisms due to competition for food in the substrate.

The dynamic equilibrium of the microorganisms present in the bed depends on its ability to adapt to the environment, which in turn will determine its greater or lesser competitiveness (TIQUIA et al., 1997). From the results obtained in T3 it is assumed that the adaptation and activity of ***Bacillus subtilis*** in avian bed depend on its initial population, since T2, which contains half of the recommended dose, did not differ from the control group.

According to BRITO & TAGLIARI, (2007) there was a marked reduction in the amount of ***Escherichia coli*** in broiler bed from 24 hours of contact of Impact-P® with the bed. In this same study the authors verified that the use of Impact-P® was able to significantly prevent the occurrence of cellulite in chickens exposed to pathogenic strains of ***Escherichia coli*** .

Table 1 - Effects of treatments and bed time after application of Impact P® on the number of CFU in a selective medium for enterobacteria ($\log_{10}$ CFU).

Treatments*	$\log_{10}$ (CFU)
T1 (control)	3.31 A
T2 (2.5g m^{-2} of bed)	3.30 A
T3 (5.0g m^{-2} of bed)	2.89 B

Bed Time (weeks) **	$\log_{10}$ (CFU)
1	2.65 A
2	3.27 B
3	3.21 AB
4	3.40 B

Treatment	prob = 0.0034
Bed Time	prob = 0.0104
Treatment x Bed Time	prob = 0.58

Means followed by different letters in the column differ significantly by the Tukey-Kramer test (P <0.05).
* Evaluation period (1 to 4 weeks).
** Data from Treatments 1, 2 and 3 analyzed statistically together to verify the effect of time of bed use.

As can be seen in table 1 , the enterobacteria count increased with bed time (P <0.05). This result suggests the need for a possible reapplication of the Impact-P® product after two weeks of use by broiler breeder dams to maintain the enterobacteria population at lower levels than when the product is not applied. In confined conditions, the diseases are directly related to the level of environmental contamination (SOBESTIANSKY, 2002). It is important to point out that in the case of the matrices, a daily load of very large wastes was added to the bed, in addition to feed and water.

Table 2 shows the chicken bed enterobacteria counts after counting on McConkey agar. Again the results indicate the superiority of the treatment with 5.0 g m $^{-2}$ of Impact-P® for the reduction of the microbial population in the aviary bed, among them, possibly ***Escherichia coli*** and ***Salmonella*** .

Table 2 - Effects of the application of Impact P® on avian bed on the number of colonies in a selective medium for enterobacteria McConkey (log_{10} CFU).

Treatments*	Lactose positive	Lactose negative
	log_{10} (CFU)	log_{10} (CFU)
T1 (control)	1.05 A	1.60 A
T2 (2.5g m^{-2} of bed)	1.15 A	1.56 A
T3 (5.0g m^{-2} of bed)	0.68 B	1.15 B

Averages followed by different letters in column differ significantly by the Tukey-Kramer test (P <0.05).
*Averages of bed time evaluation (weeks 1, 2, 3 and 4).

It is concluded that the application of Impact-P® in the dosage of 5 g m $^{-2}$ bed significantly reduces the number of bacteria in the bed of broiler chicken matrices.

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REFERENCES
AVILA, VS et al. **Avian bed: materials, reuse, use as food and fertilizer** . Concordia, Brazil: EMBRAPA-CNPSA, 1992. 90p. [Links]
BRITO, BG; TAGLIARI, KC Effect of the use of Impact-p on the occurrence of cellulite in broilers. **The Veterinary Hour** , Porto Alegre, v.26, n.155, p.13-20, 2007. [Links]
CHERNAKI-LEFFER. AM et al. Isolation of Enterobacteriaceae in ***Alphitobius diaperinus*** and aviary beds in the western Paraná State, Brazil. **Revista Brasileira de Ciência Avícola** , Campinas, v.4 n.3, p.243-247, 2002. [Links]
KIEHL, EJ **Composting Manual** . 4.ed. Piracicaba: Ceres, 2004. 173p. [Links]
MOORE, PA et al. Effect of chemical amendments on ammonia volatilization from poultry litter. **Journal of Environmental Quality** , Madison, v.24, n.2, p.293-300, 1995. [Links]
OLIVEIRA, MC et al. Dry matter content, pH and volatilized ammonia of chicken bed treated or not with different additives. **Revista Brasileira de Zootecnia** , Viçosa, v.32, n.4, p.824-829, 2003. [Links]
PROCHONOW, LI et al. Control of ammonia volatilization in composting, by addition of agricultural gypsum and superphosphate with different levels of residual acidity. **Soil Science Journal** , v.25, n.1, p.65-70, 2001. [Links]
POPE, M .; CHERRY, TE Evaluation of the presence of pathogens on broilers raised on poultry litter treatment ® -treated litter. **Poultry Science** , Savoy, v.79, n.9. p.1351-1355, 2000. [Links]
SINGH, HP et al. Effect of different methods of treatment of used litter on growth, feed efficiency and economies in broiler production. **Indian Journal Production and Management** . v.6, 109-114, 1990. [Links]
SOBESTIANSKY, J. **Intensive system of production of pigs - Biosafety program** . Goiânia, GO: Art 3. Special prints, 2002. 130p. [Links]
TIQUIA, SM et al. Effects of turning frequency on composting of spent pig-manure sawdust litter. **Bioresource Technology** , v.62, n.1, p.37-42, 1997. [Links]
WALTER, L. Broiler bed management and microbiological aspects in the production environment. In: INTERNATIONAL SEMINAR ON COCCIDIOSIS AND INTESTINAL QUALITY, 2000, Campinas, SP. **Anais ...** Campinas: COCCIFORUM, 2000. 129p. p.44-54. [Links]
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