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HAAA Workshop





Use of biotechnology to improve muscle growth in aquaculture species: Preliminary results on the use of myostatin in tilapia

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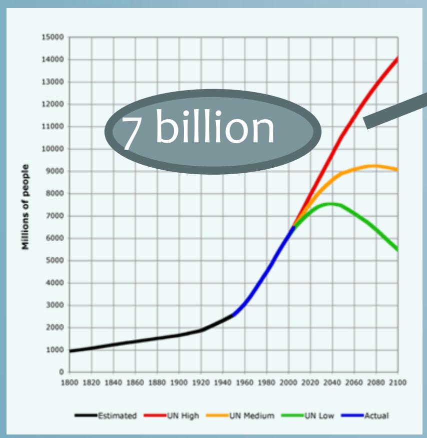
DO WE NEED ANIMAL BIOTECHNOLOGY?

How Do We Feed Growing Population?

UN 2004 projection



9 billion by 2050

Need 70% to 100% increase from the current Ag output (UN, 2002)

Options

1. Expand agricultural land
2. Increase the yield (production efficiency)

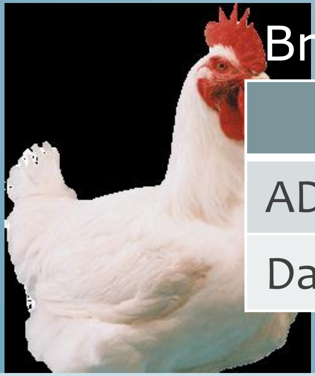
concern about the impact of animal agriculture on environment (water quality, global warming etc,)

New Development of Technologies to Improve the Efficiency of Meat Animal Production including fish

Changes in animal production efficiency

Changes in the efficiency of pig production

	1951	1998
# of pigs/sow	6.2	19
Kg feed/gain	8.0	3.2



Broiler weight gain

	1980	1990	2000	2010
ADG (g)	27	38	49	59
Days to 2.2 kg	81.5	57.9	44.9	37.3

Will this change continue? – probably so, but with much less magnitude

Need for new technology: our knowledge on animal growth process (skeletal muscle growth process) in combination with biotechnology can contribute to developing new methods of improving skeletal muscle growth.

Skeletal muscle growth

Positive growth factors

Ex. GH, IGF-I & II, steroids etc

Negative growth factors

Ex. Myostatin

Use of myostatin to imporve meat production

- *Myostatin?*
  - *Identified in 1997*
  - *Potent negative regulator of skeletal muscle growth and development*
  - *Almost exclusive on skeletal muscle*

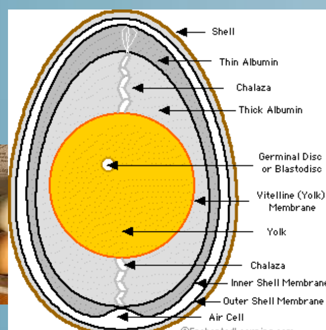
Effects of In-ovo Injection of Anti-myostatin Antibody on Post-hatch Chicken Growth and Muscle Mass

Y S KIM, N K BOBBILI, K S PAEK AND H J JIN

POULTRY SCI. 85:1062-1071 (2006)

Injection: 40 µg/50 µl PBS at 3 d after incubation

Areas of injection: yolk



Hatchability and number of birds sacrificed at 5 wk after hatch

Parameters	Control	Injection
Number of eggs incubated	40	60
Number of eggs hatched	36	40
% hatchability	90%	67%
Number of birds raised until sacrifice	36	36
Number of male	19	14
Number of female	17	22

Body weight of broiler chickens at different age as affected by *in-ovo* administration of anti-myostatin antibody

	Treatment		Sex	
Age, d	Con	Injection	Male	Female
1	46.3	45.1	45.5	44.9
7	147.7	161.1	153.1	152.8
14	457.0	488.5	477.7	454.0*
21	891.5	943.2	942.3	872.8*
25	1209.1	1252.2	1257.0	1163.6*
28	1394.9	1445.6	1456.0	1346.3*
35	1778.7	1854.0*	1844.6	1712.8*

Data are least square mean (SEM).
*, P<0.05;

Carcass and organ weights of broiler chickens as affected by *in-ovo* administration of anti-myostatin antibody

	Treatment		Sex	
	Control	Injection	Male	Female
35 d body wt, g	1778.7	1853.9*	1844.6	1712.8*
Carcass wt, g	1313.0	1374.7*	1358.1	1268.0*
Dressing %	73.5	74.1	73.6	74.1
Leg wt[1], g	357.1	376.9*	374.2	340.0*
Breast muscle wt, g	286.1	301.8+	280.6	291.5*
Liver wt, g	43.4	45.4	43.4	43.4
Heart wt, g	8.21	8.75*	8.69	7.74+
Spleen wt, g	1.49	1.57	1.57	1.40
Abdominal fat, g	35.9	41.1*	35.3	36.4

Data are least square mean (SEM).
*, P<0.05; +, P<0.1
[1]Includes thigh and leg with bone-in.

Feed consumption and feed efficiency

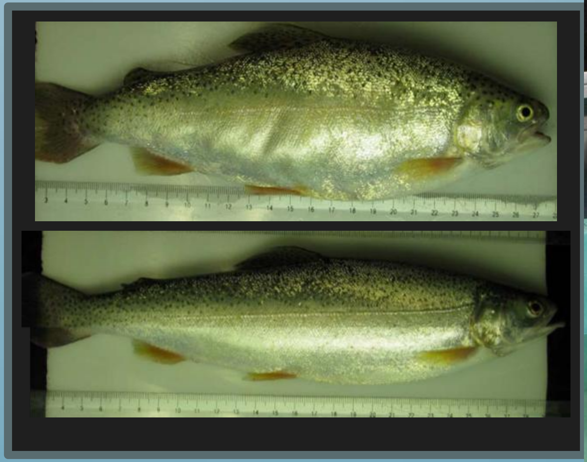
	Control	Injection
Average weight gain (10-35 day)	1651.6	1798.2
Average feed intake (10-25 day)	2810.4	2600.2
Gain/feed ratio	0.59	0.69

Myostatin inhibition to improve fish growth

Case 1) Use of follistatin, a myostatin inhibitor

Erika et al. Overexpression of follistatin in trout stimulates increased muscling. (2009) Am. J. Physiol.

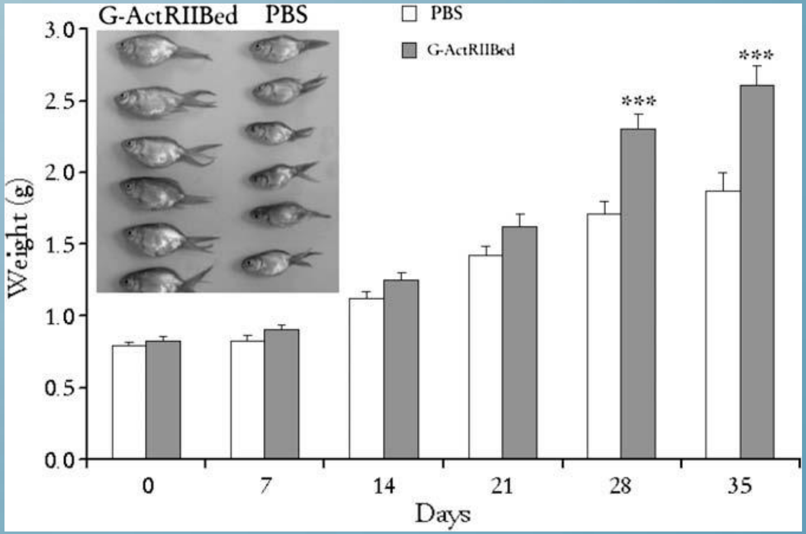
Transgenic overexpression of protein (follistatin) suppressing myostatin



Case 2) Use of ActRIIB-ECD, a myostatin inhibitor

Carpio et al., Regulation of body mass growth through activin type IIB receptor in teleost fish.
General and Comparative Endocrinology 160 (2009) 158–167

- production of Recombinant protein (ActRIIB-ECD) suppressing myostatin
- Immersion bath method

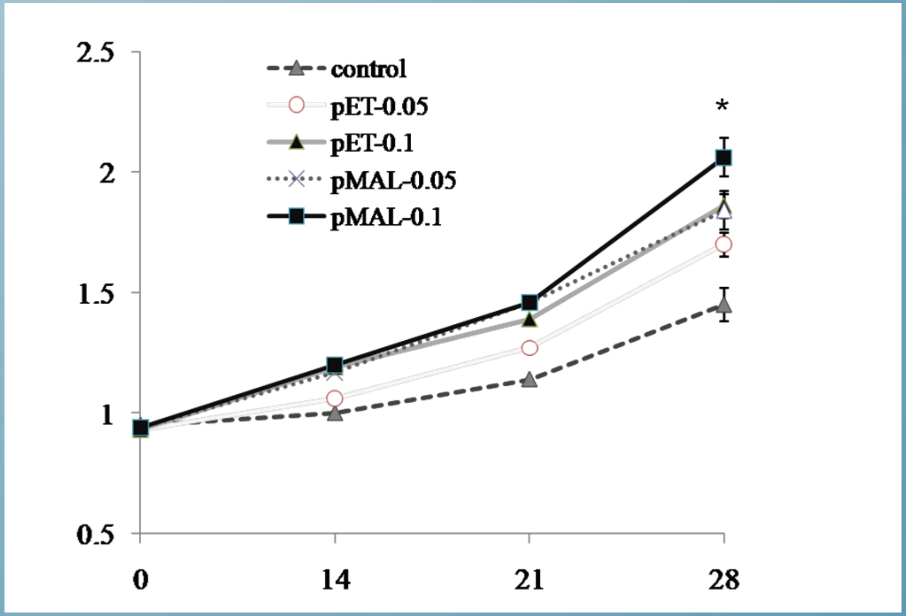


- *Improve the growth of goldfish, tilapia, and African catfish*

Case 3) Use of prodomain, a myostatin inhibitor

Lee et al., 2010. Improving rainbow trout (*Oncorhynchus mykiss*) growth by treatment with a fish (*Paralichthys olivaceus*) myostatin prodomain expressed in soluble forms in E.coli. (2010) Aquaculture 302:270-278.

- production of Recombinant protein (prodomain) suppressing myostatin
- Immersion bath method



Recent trial (2010-2011) with tilapia in Hawaii

- *Objective*
  - *To examine whether the enhanced weight gain induced by treatment with myostatin inhibitors at early stages of fish growth would lead to heavier market weight. This current study was designed to address this question.*

- Experimental group allocation:
  4 groups (no immersion, buffer only, 0.05 mg/L and 0.2 mg/L)

- Treatment:
  For 4 weeks twice a week
  Weighing by group

- Immersion procedure:
  2 hours in 200 ml solution containing myostatin prodomain

Table 1. Effects of immersion bath with fish (*P. olivaceus*) myostatin prodomain on tilapia growth during the 1^{st} phase before moving into a large tank

Week after bath immersion	Treatment groups[1]				P value
	No bath	Bath 1 (0 mg/L)	Bath 2 (0.05 mg/L)	Bath 3 (0.2 mg/L)	
0 day	(n=150)	(n=150)	(n=150)	(n=150)	
Wt, mg	111.9	111.5	112.8	110.1	NS
4 week	(n=138)	(n=141)	(n=134)	(n=145)	
Wt, mg	224.8	218.0	237.3	231.3	0.07
6 week	(n=133)	(n=138)	(n=131)	(n=145)	
Wt, mg	459.0[a]	473.4[a]	506.7[b]	507.8[b]	0.002
Length, cm	2.34[a]	2.41[b]	2.45[b]	2.46[b]	0.000
CF[2]	3.53[a]	3.33[b]	3.40[b]	3.36[b]	0.002
14 week	(n=96)	(n=101)	(n=92)	(n=111)	
Wt, g	9.29[a,b]	9.78[b]	10.34[b]	8.52[a]	0.001
Length, cm	6.38[a,b]	6.47[a,b]	6.50[b]	6.21[a]	0.037
CF[2]	3.42[a]	3.51[a,b]	3.57[b]	3.44[a]	0.010
19 week	(n=96)	(n=101)	(n=92)	(n=110)	
Wt, g	40.01[a,b]	40.39[a,b]	43.04[b]	36.74[a]	0.001
Length, cm	10.14	10.13	10.32	9.90	0.208
CF[2]	3.63	3.73	3.70	3.63	0.183

Data are least square mean ± (SEM); SEMs of 0 day wt are from 3 tanks and SEMs of the rest are from individual weights.

[1] Mean difference was analyzed using Fisher's pairwise comparisons. Means in the same row not sharing the same superscript differ at $p<0.05$.

[2] Condition factor = (body wt/$length^3$) x 100

Treatment showed significant effect on body mass during earlier treatment , but not effect on later stage after treatment

Table 2. Effects of immersion bath with fish (*P. olivaceus*) myostatin prodomain on tilapia growth during the 2^{nd} phase after transfer to a large tank – male and female together (5 females)

Wk after bath immersion	Treatment groups[1]				P value
	No bath	Bath 1 (0 mg/L)	Bath 2 (0.05 mg/L)	Bath 3(0.2 mg/L)	
23 week (07/22/10)	(n=18)	(n=18)	(n=18)	(n=18)	
Wt, g	70.4[a,b]	75.1[a,b]	77.6[a]	65.4[b]	0.01
Length, cm	12.47[a,b]	12.72[a]	12.68[a]	12.07[b]	0.021
CF[1]	3.60	3.63	3.80	3.71	0.148
32 week (09/17/10)	(n=16)	(n=17)	(n=18)	(n=17)	
Wt, g	153.2	153.5	171.7	173.5	0.230
Length, cm	16.47	16.46	16.98	17.11	0.202
CF[1]	3.37	3.43	3.46	3.40	0.872
42 week (11/30/10)	(n=16)	(n=17)	(n=18)	(n=16)	
Wt, g	264.9	297.2	290.9	311.1	0.117
Length, cm	19.51	19.94	19.92	20.48	0.153
CF[1]	3.51	3.71	3.65	3.60	0.342
45 week (12/20/10)	(n=16)	(n=17)	(n=18)	(n=15)	
Wt, g	271.9	306.7	299.4	319.5	0.137
Length, cm	20.63	21.29	21.14	21.60	0.236
CF[1]	3.05	3.17	3.14	3.14	0.691

Data are least square means ± (SEM). [1]Mean difference was analyzed using Fisher's pairwise comparisons. Means in the same row not sharing the same superscript differ at $p<0.05$.
[1]Condition factor = $(\text{body wt}/\text{length}^3) \times 100$

No treatment effect was observed on body mass at market size.

Table 3. Effects of immersion bath with fish (*P. olivaceus*) myostatin prodomain on body weight, length, and organ weights of male tilapia

Parameters	Treatment groups				P value
	No bath (n=13)	Bath 1, 0 mg/L (n=17)	Bath 2, 0.05 mg/L (n=16)	Bath 3, 0.2 mg/L (n=15)	
Wt, g					
Length,					
CF[1]					
Empty					
% empt					
Liver w					
% liver					
Heart w					
% heart					
Fillet w					
% fillet					

Data ar
[1]Condit
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Summary of tilapia preliminary experiment

- Tilapia growth rate was improved during the prodomain treatment at larval stage.
- The improved growth disappeared at later stages of growth after treatment, resulting in no treatment effect on body and fillet wt at market size.

Limitations in the preliminary experiment

- Species compatibility of prodomain (flat fish prodomain vs tilapia prodomain)
- Sample size
- Effect of stages of treatment



Mahalo!

Questions?