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AN INDUSTRIAL–SCALE STUDY ON THE IMPACT OF VACCINATION UPON RAINBOW TROUT PERFORMANCE

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Summary

The effect of intraperitoneal (ip) vaccination upon the performance of rainbow trout (n = 1611), maintained under commercial production conditions (aerated spring water, 9.8 °C, 150 L/min), was evaluated over a 7–week period. Vaccine impact was examined with reference to control (n = 1683) and injected control (sterile filtered water; n = 1537) animals. All groups were run in triplicate (*i. e.,* n ≥ 500 fish per gorup). Animals were fed to satiation twice daily. Vaccination suppressed (P <0.05) growth in weight over the entire period of study when compared to control treatments. A corresponding decline in specific growth rates (P < 0.05), over the first 29 d of the trial, was also observed for vaccinated fish. Feed conversion efficiencies and feed ratio were similarly negatively affected in vaccinated animals for 29 d post–vaccination (P <0.05). The vaccine caused abdominal adhesions although no differences were observed in body composition.

Key words: rainbow trout, adhesions, furunculosis, growth, vaccine, vibriosis

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INTRODUCTION

Increased occurrence of bacterial and other diseases are often associated with intensification in animal production systems, where farmed inventory may be frequently stressed by adverse conditions (*e. g.* inadequate nutrition, overcrowding, hierarchy development, etc.). This is particularly the case during the industrial cultivation of teleosts. Here, a combination of factors, including environmental stresses (Strunjak–Perović *et al.,* 1995; Iwama *et al.,* 1997), the lack of domesticated strains and insfufficient knowledge of the biological requirements of farmed species (McLean and Devlin, 1999), and the prevalence of microbes in the water column (Woo, 1992), often unite to create devastating economic losses following the outbreak of disease. Not surprisingly, effective control of epizootics has become vital to success in aquaculture.

Several bacterial and fungal diseases of fish have been successfully controlled by chemostats, although such chemicals have caused environmental concerns (GESAMP, 1996). For example, apprehension has been expressed with regard to antibiotic build–up in edible portions of fish flesh (Austin, 1993). Similar fears have accompanied the persistence of asymptomatic carriers of disease (*e. g.* furunculosis; Ortega *et al.,* 1996). Carriers can cause epizootics when transferred to other livestock following, for example, grading or stock transfers.

Disease control by vaccination has been successfully applied to combat various fish pathogens (*e. g. Listonella anguillarum, Aeromonas salmonicida*, and *Yersenia ruckeri*; see: Newman, 1993). And, commercial application of vaccines by the salmonid culture industry has resulted, not only in significant reductions in mortalities and disease–associated financial loss to the industry, but also substantial declines in the use of antibiotics (GESAMP, 1996). Nevertheless, while vaccination represents a major advance in the control of specific diseases, treatments may be stressful and cause detectable side–effects in cultured fish and other animals (*e. g.,* Dohoo and Montgomery, 1996).

The most efficaceous method for delivering vaccines to fish remains by injection (Horne and Ellis, 1988). This route of delivery, as opposed to immersion and oral vaccination, for example, provides advantage in that it permits the simultaneous delivery of adjuvants that stimulate the immune response (review: Audibert and Lise, 1993). However, following vaccination, animals exhibit stress–related symptoms that include decreased appetite and poorer feed conversion efficiencies (Lillehaug, 1991; Lillehaug *et al.,* 1992). In addition, vaccines have been associated with changes to abdominal lumen structure and injection site infections. These latter effects have the potential to cause a downgrading of the end product (Hoel and Lillehaug, 1997) and loss of income to the farmer (Rønsholdt and McLean, 1999). However, comparatively few studies have examined the effect of vaccination upon fish at an industrial scale such that it remains difficult to draw firm conclusions relating to the possible negative effects of vaccines, particularly where challenges are not given.

Accordingly, the present study was initiated in order to examine the overall impact of injection vaccination upon various performance characteristics of rainbow trout reared under commercial conditions. Attention focused upon the effect of vaccination upon growth and feed conversion efficiencies and the impact of treatment upon body composition and physical attributes. The ultimate objective of the trial was to provide information that the fish farmer could employ as guidelines with respect to managing vaccinated animals. Since use of vaccines represents an important decision–based process, a further aim of the study was to provide data to assist the aquaculturist in cost: benefit analysis prior to implementing vaccination programmes.

MATERIALS AND METHODS

Animals and husbandry

The present trial was undertaken at an industrial aquaculture facility (Ribogojilište »Krčić«, Knin, Croatia). Rainbow trout, *Oncorhynchus mykiss* (23.4 ± 2.9 g wet wt.; N = 4831) were randomly divided into three treatment groups *viz.* control (n = 1683), injected control (n = 1537) and vaccinated (n = 1611). Following random distribution, groups were further divided into triplicates (n ≥ 513 per group) and assigned to one of nine indoor concrete raceways (L x W x [H_1 x H_2]; 7.06 x 0.97 x [0.435 x 0.348] m.). Each raceway was supplied with aerated (9.8 ± 0.06°C) spring water (150 L/min.). Animals were fed to satiation twice daily (SAFIR, Aller Mølle, Denmark. 45% protein, 20% lipid, 8% ash), except for the day before weighing. Raceways were subjected to a natural photoperiod.

Treatments

Groups were allowed to acclimate to experimental conditions for a period of 2 wk prior to initiation of the study. Control groups were either left untreated or received a single injection of 200–μL sterilised filtered (Merrild KF No. 3) water. Vaccinated animals were given single ip injections (200–μL) of Apoject® 1800_{VET} (a bivalent, oil–based adjuvant, Vibriosis, *Listonella anguillarum* [formerly *Vibrio anguillarum*] serotype 01 and 02 and Furunculosis, *Aeromonas salmonicida* ssp. *salmonicida* vaccine; Alpharma AS, Norway).

Analytical procedures

Following experiment start, animals were weighed bimonthly over an 8 wk period. Weight specific growth rate (SGR; %/day) was calculated according to the equation:

$$SGR = (\{ln\ W_2 - ln\ W_1\}/\{t_2 - t_1\}) \times 100,$$

Where: *ln* [W_2] and *ln* [W_1] were the natural logarithms of weight at the end (t_2) or start (t_1) of the time interval respectively (Weatherley and Gill, 1987). Feed conversion efficiencies (FCE) and feeding ratio (FR), for each treatment, was calculated as described by Cowey (1992). Proximate composition of whole fish was evaluated prior to experiment start and following trial termination according to the procedures outlined in Teskeredžić *et al.* (1995). Determinations were performed in triplicate upon eight randomly taken fish per treatment group. The severity of external lesions and abdominal adhesions were evaluated 7 wk. postvaccination for all groups using the methods outlined in Midtlyng *et al.* (1995). From each treatment 25 fish were randomly taken and killed by cranial fracture. Using an individual who was blind to treatment code, fish were examined and graded according to descriptors presented in Table 1. Subsequently, animals were opened by a ventral cut from anus to gills and evaluated for overall appearance in accordance with the scheme presented in Table 2.

Statistical analyses

Statistical analysis of weight, SGR, FCE and chemical composition were performed using a one–factor design (Montgomery, 1997).

The model applied was:

$$y_{ij} = \mu + A_i + \varepsilon_{(i)j},$$

where: μ was the true mean, A_i the treatment effect, and $\varepsilon_{(i)j}$ the residual (random effect). Analyses were performed using SigmaStat (v. 1.0, Jandel Corporation). Between tratments, with normal distribution and equal variance, one–way Analysis of Variance (ANOVA) was used to test for homogenerity, while Student–Newman–Keul's method was employed in isolating differing treatments. Significance differences were determined using a 95% level.

RESULTS

Mortalities were recorded at 1.0%, 0.9% and 5.65% for control, control injected and vaccinated fish respectively. High mortality was observed for the vaccination group, particularly over the first 2–h post–treatment. Due to fish loss following tank rupture and subsequent associated modifications to feeding and behavioural (aggression, etc.) characteristics of a control injected group, results from this treatmen were not included in further evaluations. Figure 1 summarises the weight growth performance of the three treatment groups. Significant differences ($P<0.05$) were found over the 50–day period of observation, with vaccinated fish returning inferior growth. At the first weighing point, 2 wk into the trial, vaccinated animals were 11% and 9% smaller than untreated and injected control groups respectively ($P<0.05$). This trend

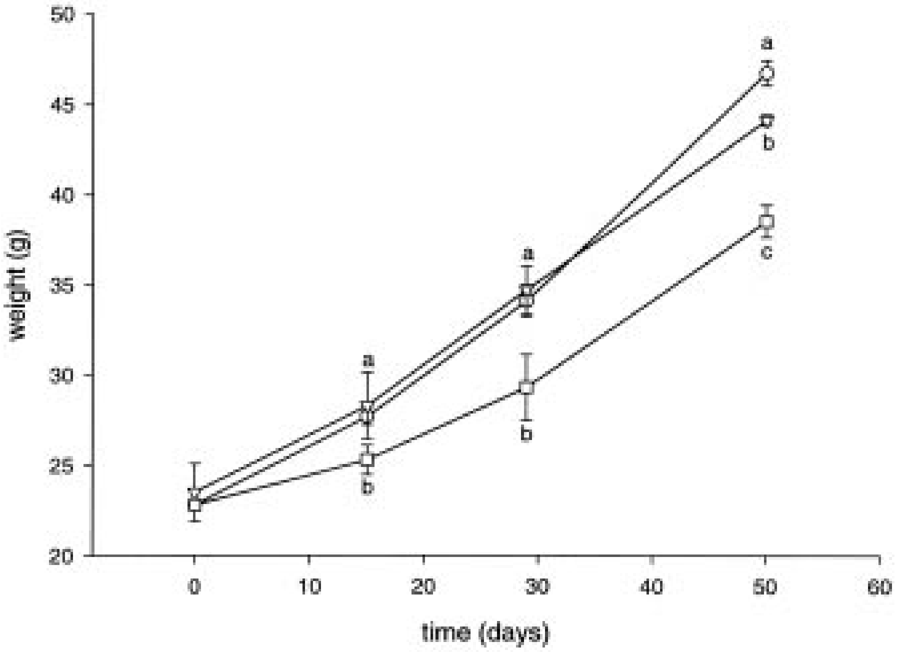

Figure 1. Mean (±95% confidence limits) growth performance of control (❍), injected control (v), and vaccinated (☐) rainbow trout over a 50 day trial period. Each group represents the pooled data of triplicate treatments (n ≥ 1500 per treatment). Different letters identifies differences between group weights (P < 0.05)

Slika 1. Srednje vrijednosti rasta kontrole (❍), injicirane kontrole (v) i vakciniranih (☐) pastrva kroz vrijeme od 50 dana. Svaka grupa predstavlja skupne podatke trostrukog tretmana (n ≥ 1500 po tretmanu). Različita slova upućuju na razlike između grupa u težini (P < 0,05).

continued until trial end (Fig. 1), at which point a significant weight divergence was noted between the two control groups with the injected control fish differing in weight by 6% (P<0.05) and vaccinated animals weighing 21% less than unmanipulated controls (P<0.05; Fig. 1).

Table 4 presents the weight SGR of experimental treatments. Significant differences (P<0.05) in wSGR were recorded between vaccinated and control groups for the periods day 0–15 and day 15–29, representing approximately 45% and 30% reductions respectively. From day 29 onwards however, no differences in SGRs were recorded between treatments. Evaluation of SGR throughout the entire trial period indicated that vaccination significantly (P<0.05) reduced wSGR by 37% when compared to unmanipulated controls and by 18% when matched against injection control trout. wSGRs throughout the trial were 1.28±0.04 (controls) > 1.10±0.30 (control injected) > 0.94±0.07 (vaccinated).

Table 1. Scheme employed to evaluate the severity of externa lesioning on experimental rainbow trout following various treatments

Tablica 1. Shematski prikaz procjene nekih vanjskih lezija na eksperimentalnim pastrvama kod raznih tretmana

Score	Visual appearance of external state
0	No visible pathological alterations due to vaccination at the injection site, no visible external wounds or scarring.
1	Lesions visible at the injection site together with wounds and small scars observed on the fin, skin and gills
2	Swollen lesions and bursts at the injection site, and/or visible signs of injection site infection. Numerous scars on both fins and skin. Skin covered by a thick mucus layer.

Table 2. Scheme employed to evaluate the severity of abdominal adhesions of experimental rainbow following various treatments.

Tablica 2. Shematski prikaz procjene nekih abdominalnih adhezija na eksperimentalnim kalifornijskim pastrvama kod raznih tretmana.

Score	Visual appearance of abdominal cavity
0	No adhesion or visible pathological changes due to vaccination
1	A few thin fibres generally appearring between the place of inhection and pyloric cacae/spleen. These fibres were easy to loosen/tear. Sometimes combined with small melanin (dark spot) deposits in the abdominal wall and adipose tissue firmly attached to the spleen.
2	Several fibres covering a larger area, some of which were thicker and more developed than described above, but easy to loosen.
3	Marked adhesions between ventral organs and abdominal wall, easily loosened. Sometimes combined with melanin deposits in the abdominal wall.
4	Extensive and numerous adhesions covering larger areas. Considerable force need to loosen adhesions. Sometimes combined with melanin deposits in the abdominal wall.
5	Ventral organs more or less grown together. Extensive lesions difficult to loosen. Usually combined with melanin deposits in the abdominal wall together with some hyperaemia.
6	As for 5, but even more distinct and extensive. Bursts and wounds visible when adherions were loosened. Usually combined with greater levels of melanin deposits in the abdominal wall and severe hyperaemia.

The impact of different treatments upon proximate composition of control and vaccinated trout are described in Table 3 for fish of equal weight ($P = 0.32$) and length ($P = 0.66$). Relative to start values, all groups of trout expressed significant ($P < 0.05$) declines in protein (~18%) and lipid (~33%) levels. However, values for percent ash and moisture remained similar. Vaccination, and hence differing gowth rate (Fig. 1, Table 3) did not impact overall body composition of experimental animals (Table 3).

Feeding ratio of vaccinated rainbow trout differed ($P<0.05$), from injection control and control treatments, by 23% and 24% respectively over the first 15

Table 3. Proximate composition (± 95% confidence limits) of day 0, control, injected control and vaccinated rainbow trout 57 days following trial initiation. Different letters identify differences between treatment groups (P <0.05; Student–Newman–Keuls).

Tablica 3. Kemijski sastav kalifornijskih pastrva na početku pokusa, te kontrole, injicirane kontrole i cijepljenih riba nakon 57 dana pokusa. Različita slova označuju razlike između tretiranih grupa (P <0,05); Student–Newman–Keul test).

Time	Treatment	Protein [%]	Lipid [%]	Ash [%]	Moisture [%]
Day 0	Prior	19.74 ± 0.31[a]	7.41 ± 0.58[a]	2.16 ± 0.21[a]	70.61 ± 0.61[a]
Day 57	Control	16.65 ± 0.58[b]	11.69 ± 0.58[b]	3.17 ± 1.36[a]	69.47 ± 0.17[a]
	Injection control	16.33 ± 0.71[b]	11.83 ± 0.98[b]	2.40 ± 0.91[a]	69.45 ± 1.09[a]
	Vaccination	16.41 ± 0.36[b]	10.64 ± 0.81[b]	2.57 ± 0.27[a]	70.46 ± 0.67[a]

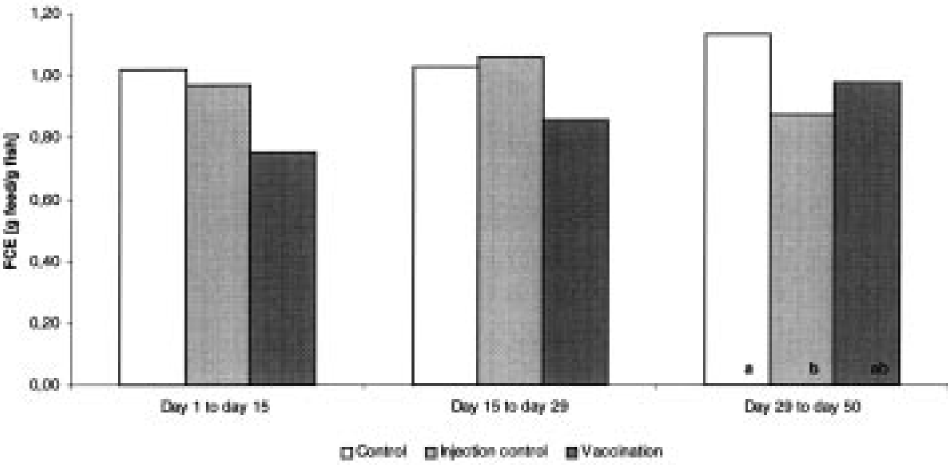

Figure 2 Mean values for feed conversion efficiencies of control (□), injected control (▒), and vaccinated (■) rainbow trout over 50 day trial period. Each group represents the pooled data of triplicate treatments (n ≥ 1500 per treatment). Different letters identify differences (p <0.05; Student-Newman-Keuls test) between groups, calculated using the pooled standard variation for three treatments

Slika 2. Srednje vrijednosti konverzije hrane kontrole (□), injicirane kontreole (▒) i vakciniranih (■) riba kroz vrijeme pokusa od 50 dana. Svaka grupa predstavlja skupne podatke trostrukog tretmana (n ≥ 1500 po tretmanu). Različita slova ukazuju na razlike (p <0.05; Student–Newman–Keulov test) između grupa, izračunano primjenom skupne standardne varijacije za tri tretmana

Table 4. Weight specific growth rate and feeding ratios (± 95% confidence limits) of control, injected control and vaccinated rainbow trout for specific time points throughout the trial. Different letters identify differences between treatment groups (P<0.05 ; Student–Newman–Keuls).

Tablica 4. Dnevni prirast težine i količina obroka (±95% granice pouzdanosti) kontrole, injicirane kontrole i vakciniranih kalifornijskih pastrva kroz vrijeme pokusa. Različita slova označuju razlike između tretiranih grupa (P <0,05; Student–Newman–Keulov test).

	Treatment	Day 1 to day 15 [% day^{-1}]	Day 15 to day 29 [% day^{-1}]	Day 29 to day 50 [% day^{-1}]
Specific growth rate	Control	1.38±0.19^{a}	1.49±0.13^{a}	1.50±0.19^{a}
	Injection control	1.29±0.08^{ab}	1.47±0.51^{a}	1.10±0.40^{a}
	Vaccination	0.74±0.38^{b}	1.04±0.36^{a}	1.31±0.31^{a}
Feeding rate	Control	1.23±0.06^{a}	1.31±0.12^{a}	1.14±0.11^{a}
	Injection control	1.22±0.08^{ab}	1.25±0.10^{a}	1.13±0.38^{a}
	Vaccination	0.94±0.07^{b}	1.14±0.07^{a}	1.17±0.05^{a}

Table 5. Symmary of the distribution of internal and external damages to 24 randomly graded fish taken from one of three experimental groups. The numbers in the table indicate the frequency of the score in each treatment. Grading was performed upon randomly taken tag coded fish that was unknown to the grader. See Tables 1 and 2 for further details.

Tablica 5. Skupni prikaz vanjskih i unutarnjih oštećenja na 24 odoka uzetih riba iz jedne od triju eksperimentalne grupe. Broj u tablici znači učestalost pojava za svaki tretman. Za dalje detalje vidi Tablice 1 i 2.

	External			Internal				
Treatment	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2	Score 3	Score 4
Control	23		1	21	3			
Injection control	24	1		19	6			
Vaccination	22	1		6	13	2		2

days of the trial (Table 4). Between day 15–29, only control trout returned significantly (P <0.05) higher FR compared against vaccinated fish. From day 29–50, all groups performed equally with respect to FR. Feed conversion efficiencies of the three treatment groups are displayed in Fig. 2. The figure depicts three discrete periods of the trial. During the first 24 days of the experiment vaccinated fish had a 30% higher FCE when compared to the control treatment, whereas the injection control treatment returned a FCE that was 26% lower than the vacination treatment and 5% higher than control unmanipulated animals (Fig. 2). Between day 15–29, control trout yielded a

FCE 19% lower than that recorded for vaccinated fish. In contrast, the injected controls presented a FCE that was 21% lower than the vaccinated group and 3% lower than controls (Fig. 2). From day 29 until the end of data acquisition on day 50, FCEs were similar for vacinated and injected controls, with the latter fish performing below the level achieved by the untreated controls.

Intra–abdominal adhesions were observed in both injected control and vaccinated fish (Table 5). However, it was only with vaccinated animals, and with a greater frequency, that more serious internal adhesion was recorded. By 50–d post–ip injection, however, external lesions were generally not visible (Table 5). The majority of internal adhesions were placed in the area immediately surrounding the injection–site.

DISCUSSION

The present study represents the first industrial–scale investigation of the effects of vaccination upon the performance characteristics of rainbow trout. Unlike several previous studies that have evaluated the impact of vaccines on farmed salmonids (*e.g.*, Midtlying *et al.,* 1995, 1996), the trial undertaken here did not present fish with artificial challenge(s). This enabled a more definitive evaluation of the effects of vaccination upon important production––related processes without the masking effects of a disease process. Moreover, most previous studies with vaccines have employed experimental, rather than commercially available formulations, which limits the practical usefulness of acquired data.

The major consequence of treating rainbow trout with vaccine was a clear–cut and sustained growth depression. Similar effects have been noted for rainbow trout of greater initial start size (90g; Rønsholdt and McLean, 1999). Interestingly, irrespective of original size, over a similar time span, vaccination caused identical weight growth penalties (21%). Examination of SGR from the present study indicates that treatment suppressed growth for a period of at least 29 days post–vaccination, after which values matched those of controls. Nevertheless, given that treated fish were of lower mean size growth parity was not achieved in real terms. In previous studies, SGRs have been reported to normalise after approximately 14 days, coinciding with a return of appetite (Midtlyng *et al.*, 1995; Rønsholdt and McLean, 1999). Other trials however, harmonise with the present findings. Thus, Kitlen *et al.* (1997) and Hoel and Lillehaug (1997), observed suppressed growth for 4 weeks following vaccination of rainbow trout and Atlantic salmon respectively. The noted discrepancies between the findings of the present and latter studies may occur due to variations in nutrition, reflect strain–, or species–dependent responses, or accent the complexities inherent in feeding animals evenly to satiation at the industrial scale compared to laboratory–based. experiments. The latter supposition has some credence since comparisons of FCE between studies indicate, on average, poorer conversion rates at the receway level.

Correlating with FCE, feeding ratio was significantly reduced for at least 29 days post–vaccination. This poorer efficiency of conversion probably resulted due to loss of appetite following vaccination that might be considered to result due to the onset of a moderate »infection« state. Indeed, the results presented with respect to FR and FCE compare favourably with parasitemia–associated anorexia in trout (*e. g.,* Beamish *et al.,* 1996). The growth penalty experienced by vaccinated fish has often been attributed to the adjuvant component. However, recent research indicates that formalin–killed cells, which formed the immunogenic agent of the vaccines used herein, have even greater impact (Rønsholdt and McLean, 1999). A clearer understanding of the relationship between growth suppression, appetite and vaccination however, will require further research.

A number of investigations have commented upon the formation of so–called intra–abdominal adhesions in salmonids following vaccination (*e. g.,* Midlyng *et al.*, 1995, 1996; Rønsholdt and McLean, 1999). These lesions include granulomatous tissues that adhere to visceral organs in a manner that might compromise their normal function. Histologically, adhesive tissues are characterised by the presence of high numbers of eosinophilic granule cells and granulomas implanted in fibrous tissue (Poppe and Breck, 1997). An interesting use of vaccine–induced intra–abdominal adhesions has been used as a marker to distinguish wild from hatchery–reared salmon (Lund *et al.*, 1997). The presence of intra–abdominal adhesions may cause a downgrading in value of farmed salmon (Lillehaug, 1991; Midtlyng *et al.*, 1996; Midtlying, 1996) and create difficulties during brood egg collection (Anonymous, 1996). Commercially speaking therefore, these side effects are undesired. Following necropsy, vaccinated fish of the present study exhibited abdominal adhesions in the majority of specimens. However, it is believed that any downgrading resulting from this occurrence would be limited since the adhesions were not severe. It is noteworthy that the severity of adhesioning observed contrasted to the observations of others. Thus, Hoel and Lillehaug (1997) reported harsh abdominal adhesions for Atlantic salmon while Rønsholdt and McLean (1999), concluded that the degree of adhesion severity in vaccinated rainbow trout was much higher. These differing results may be explained by the use of vaccines of contrasting formulae and species, or reflect vaccination procedures.

Another possible side effect of vaccination, that might conceivably induce a downgrading in end product value, relates to changes in body composition. This possibility has not been examined previously, although it has been established that differences in growth rate may result in changes to lipid and protein dynamics in fish (Rønsholdt, 1995). However, vaccination did not alter compositional characteristics, such that quality downgrading would not be anticipated.

Vaccination clearly causes growth penalty in salmonids (Lillehaug, 1991; Kitlen *et al.*, 1997; Hoel and Lillehaug, 1997; Rønsholdt and McLean, 1999; this study), an effect that in all likelihood extends to teleosts in general. Associated with growth depression are potentially similar forfeits with respect to downgrading. These drawbacks must, therefore, be taken into account against the protection afforded by vaccines and the risks associated with exposure to pathogens when contemplating vaccination from a commercial perspective. Where the risk of disease is high then the added insurance that vaccines provide must be considered as a cost benefit and *vice versa*. It is important to note that while fish are albe to express growth spurts following periods of under–nutrition (see: Chiristensen and McLean, 1998), this so–called compensatory mechanism never regains lost growth potential. Hence, weight loss, as observed in the present and similar investigations (*op cit.)*, represents lost profit potenital. Obviously this factor would only be of significance in regions that are not predisposed to disease.

Sažetak

UTJECAJ CIJEPLJENJA NA RAST KALIFORNIJSKE PASTRVE U KOMERCIJALNOJ PROIZVODNJI

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U pokusu koji je trajao 7 tjedana procijenio se je učinak intraperitonealnog (i/p) cijepljenja na rast kalifornijske pastrve (n = 1611) koje su držane u uvjetima komercijalne proizvodnje (aerirana izvorska voda, temperature 9,8 °C i protoka 150 l/min). Djelovanje cjepiva ispitivano je u usporedbi s kontrolom (n = 1683) i injiciranom kontrolom (sterilna filtrirana voda; n = 1537). Sve grupe bile su u triplikatu (i. e. n >500 riba/grupi). Riba je hranjena do sitosti dva puta dnevno. U usporedbi s kontrolnim grupama, cijepljenje je utjecalo na smanjeni prirast (P<0.05) u vrijeme istraživanja. Odgovarajući pad dnevnog prirasta težine (P<0.05), kroz prvih 29 dana pokusa, također je zapažen kod cijepljenih riba. Konverzija hrane, kao i količina obroka bili su slično negativni kod cijepljenih životinja 29 dana nakon cijepljenja (P<0.05). Cjepivo je uzrokovala abdominalnu adheziju, no razlike u kemijskom sastavu tijela nisu ustanovljene.

Ključne riječi: kalifornijska pastrva, adhezija, furunkuloza, rast, cjepivo

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